JC14 Rec'd PCT/PTO 04 DEC 2001

FORM PTO-1390 (REV. 10-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		K0448/7012
		U S APPLICATION NO (If known, see 37 CFR 1 5) 09/980954
INTERNATIONAL APPLICATION NO. PCT/JP00/03639	INTERNATIONAL FILING DATE 05 June 2000 (05.06.00)	PRIORITY DATE CLAIMED 04 June 1999 (04.06.99)

TITLE OF INVENTION
CRYSTAL OF RIBOSOMAL RECYCLING FACTOR (RRF) PROTEIN AND APPLICATION THEREOF ON THE BASIS OF THREE-DIMENSIONAL STRUCTURAL DATA OBTAINED FROM THE CRYSTAL

APPLICANT(S) FOR DO/EO/US
KAJI, Akira and LILJAS, Anders

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to promptly begin national examination procedures (35 U.S.C. 371(f)).
4. ☒ The US has been elected by the expiration of 19 months from the earliest claimed priority date (PCT Article31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
   a. ☐ are attached hereto (required only if not transmitted by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☒ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(C)(5)).

Items 11. To 16. Below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
14. ☐ A SECOND or SUBSEQUENT preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☒ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C.1.821-1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☒ Other items or information:

Copy of PCT/RO/101 form w/ English Translation
Copy of PCT/RO/101 form as filed
Verification of Translation of Application as Filed (English) with Sequence Listing
Verification of Translation of International Preliminary Examination Report (English)
Copy of International Search Report
Copy of PCT/IB/301,304,308,332
Application Data Sheet under 37 C.F.R. section 1.76
Statement under 37 C.F.R. section 1.821(f) (w/ hard copy & diskette)
Applicants claim small entity size
Express Mail Label No. EL819462077US
Date Mailed: December 4, 2001

U.S. APPLICATION NO (If known, see 37 CFR 1.5) 09/980954	INTERNATIONAL APPLICATION PCT/JP00/03639	ATTORNEY'S DOCKET NUMBER K0448/7012

21. ☒ The following fees are submitted:

		CALCULATIONS PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	$1000.00	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	$860.00	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but but international search fee paid to USPTO (37 CFR 1.445(a)(2)).paid to USPTO	$710.00	
International preliminary examination fee paid to USPTO (37 CFR 1.482) But all claims did not satisfy provisions of PCT Article 33(1)-(4)	$690.00	
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)	$100.00	
ENTER APPROPRIATE BASIC FEE AMOUNT = 860.00		

Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				$860.00	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total Claims	28-20 =	8	X $18.00	$144.00	
Independent Claims	4-3 =	1	X $80.00	$80.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+$270.00	$	
TOTAL OF ABOVE CALCULATIONS			=	$1084.00	
Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by				$	
SUBTOTAL			=	$542.00	
Processing fee of $130.00 for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				$	
TOTAL NATIONAL FEE			=	$542.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate coversheet (37 CFR 3.28, 3.31). $40.00 per property			+	$	
TOTAL FEES ENCLOSED			=	$542.00	
				Amount to be: refunded	$
				charged	$

a. ☒ A check in the amount of $ 542.00 To cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ In the amount of $_____ To cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 23/2825. A duplicate of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO

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ATTORNEY'S DOCKET NO. **K0448/7012 (JRV)**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	KAJI et al.
Int'l Application No.	:	PCT/JP00/03639
Int'l Filing Date	:	5 June 2000 (05.06.00)
Earliest Priority Date	:	4 June 1999 (04.06.99)
For	:	CRYSTAL OF RIBOSOMAL RECYCLING FACTOR (RRF) PROTEIN AND APPLICATION THEREOF ON THE BASIS OF THREE-DIMENSIONAL STRUCTURAL DATA OBTAINED FROM THE CRYSTAL

Box PCT
Commissioner for Patents
Washington, DC 20231

PRELIMINARY AMENDMENT

Sir:

Please amend the application as follows:

In the Specification

Please add the following section as the first section of the specification following the title. A copy of the new section is provided on a separate page attached hereto.

Related Applications

This application is a national stage filing under 35 U.S.C. § 371 of PCT application PCT/JP00/03639, filed June 5, 2000.

In the Claims

Please cancel claims 25-27, 29-46, 50 and 51 without prejudice.

Please amend the claims as follows. Applicants have included herewith pages showing markups of the claims with insertions and deletions indicated by underlining and bracketing, respectively.

3.(amended) The method according to claim 1, wherein the RRF protein crystal is bipyramidal.

4.(amended) The method according to claim 1, wherein the RRF protein crystal has a space group $P4_12_12_1$ or a space group $P4_32_12$.

5.(amended) The method according to claim 1, wherein the RRF protein crystal has a size of $0.3 \times 0.3 \times 0.5$ mm.

6.(amended) The method according to claim 1, wherein the RRF protein crystal has respective unit lattices of a size of a=b=47.3Å and c=297.6Å.

7.(amended) The method according to claim 1, wherein the RRF protein crystal is characterized by a structure coordinate described in Table 7.

8.(amended) The method according to claim 1, wherein the RRF protein crystal is derived from Thermotoga maritima.

9.(amended) The method according to claim 1, wherein the RRF protein crystal is orthorhombic.

10.(amended) The method according to claim 1, wherein the RRF protein crystal has a space group $P2_12_12$.

11.(amended) The method according to claim 1, wherein the RRF protein crystal has a size of $30 \times 50 \times 250$ μm.

12.(amended) The method according to claim 1, wherein the RRF protein crystal is derived from strain X.

13.(amended) The method according to claim 1, wherein the RRF protein crystal is crystallized by a drop-like vapour diffusion method.

14.(amended) The method according to claim 1, wherein the RRF protein crystal is a heavy atom derivative and the crystal is any crystal of the RRF protein itself, an RRF protein mutant, an RRF protein homologue or an RRF protein co-complex.

15.(amended) The method according to claim 14, wherein the heavy atom derivative is formed by reaction of a compound selected from the group consisting of thyromethal, gold thiomalate, uranyl acetate and lead chloride.

16.(amended) The method according to claim 1, wherein the RRF protein crystal is a heavy atom derivative of platinum or mercury.

17.(amended) The method according to claim 1, wherein the RRF protein is a monomer.

18.(amended) The method according to claim 1, wherein the RRF protein is characterized by amino acid displacement according to Table 5 or Table 6.

19.(amended) The method according to claim 1, wherein a compound characterized by the chemical entity bound to the active site, accessory binding site or pocket is an inhibitor to the RRF protein.

20.(amended) The method according to claim 19, wherein the inhibitor is a competitive inhibitor, an uncompetitive inhibitor or a noncompetitive inhibitor to the RRF protein.

21.(amended) The method according to claim 1, comprising determining orientation of a ligand at the active site or accessory binding site of the RRF protein.

22.(amended) The method according to claim 1, wherein the structure coordinate is a structure coordinate of the RRF protein according to Table 7.

47.(amended) The method according to claim 1, wherein the pocket of the RRF protein is a pocket in the vicinity of C-terminal positioned on a folded part separating two domains of the RRF protein.

48.(amended) The method according to claim 1, wherein the compound inhibits binding of the RRF protein to ribosome or inhibits behavior of the RRF protein on the ribosome.

49.(amended) The inhibitor to an RRF protein, obtained by the method according to claim 19.

Remarks

Applicant has amended the specification to add priority claim information for the above-identified U.S. national stage application. Applicant has amended the claims to adjust claim dependencies, to correct typographical errors, and to reduce filing fees. No new matter has been added. Copies of the new section and amended claims are attached hereto on separate pages.

Respectfully submitted,

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Attorney Docket No. K0448/7010
Date: 4 December 2001
X12/04/01

New Section Added

Related Applications

This application is a national stage filing under 35 U.S.C. § 371 of PCT application PCT/JP00/03639, filed June 5, 2000.

Amended Claims

3.(amended) The method according to claim 1 [or 2], wherein the RRF protein crystal is bipyramidal.

4.(amended) The method according to [any one of] claim[s] 1 [to 3], wherein the RRF protein crystal has a space group $P4_12_12_1$ or a space group $P4_32_12$.

5.(amended) The method according to [any one of] claim[s] 1 [to 4], wherein the RRF protein crystal has a size of 0.3 × 0.3 × 0.5 mm.

6.(amended) The method according to [any one of] claim[s] 1 [to 5], wherein the RRF protein crystal has respective unit lattices of a size of a=b=47.3Å and c=297.6Å.

7.(amended) The method according to [any one of] claim[s] 1 [to 6], wherein the RRF protein crystal is characterized by a structure coordinate described in Table 7.

8.(amended) The method according to claim[s] 1 [to 7], wherein the RRF protein crystal is derived from Thermotoga maritima [Maritima].

9.(amended) The method according to [any one of] claim 1 [or 2], wherein the RRF protein crystal is orthorhombic.

10.(amended) The method according to [any one of] claim[s] 1, [2 and 9,] wherein the RRF protein crystal has a space group $P2_12_12$.

11.(amended) The method according to [any one of] claim[s] 1 [to 2 and 9 to 10], wherein the RRF protein crystal has a size of $30 \times 50 \times 250$ μm.

12.(amended) The method according to [any one of] claim[s] 1 [to 2 and 9 to 11], wherein the RRF protein crystal is derived from strain X.

13.(amended) The method according to [any one of] claim[s] 1 [to 12], wherein the RRF protein crystal is crystallized by a drop-like vapour diffusion method.

14.(amended) The method according to [any one of] claim[s] 1 [to 13], wherein the RRF protein crystal is a heavy atom derivative and the crystal is any crystal of the RRF protein itself, an RRF protein mutant, an RRF protein homologue or an RRF protein co-complex.

15.(amended) The method according to [any one of] claim[s 1 to] 14, wherein the heavy atom derivative is formed by reaction of a compound selected from the group consisting of thyromethal, gold thiomalate, uranyl acetate and lead chloride.

16.(amended) The method according to [any one of] claim[s] 1, [2 and 9 to 12,] wherein the RRF protein crystal is a heavy atom derivative of platinum or mercury.

17.(amended) The method according to [any one of] claim[s] 1 [to 16], wherein the RRF protein is a monomer.

18.(amended) The method according to [any one of] claim[s] 1 [to 8, 13 to 15 and 17], wherein the RRF protein is characterized by amino acid displacement according to Table 5 or Table 6.

19.(amended) The method according to [any one of] claim[s] 1 [to 18], wherein a compound characterized by the chemical entity bound to the active site, accessory binding site or pocket is an inhibitor to the RRF protein.

20.(amended) The method according to [any one of] claim[s 1 to] 19, wherein the inhibitor is a competitive inhibitor, an uncompetitive inhibitor or a noncompetitive inhibitor to the RRF protein.

21.(amended) The method according to [any one of] claim[s] 1 [to 20], comprising determining orientation of a ligand at the active site or accessory binding site of the RRF protein.

22.(amended) The method according to [any one of] claim[s] 1 [to 8, 13 to 15 and 17 to 21], wherein the structure coordinate is a structure coordinate of the RRF protein according to Table 7.

47.(amended) The method according to [any one of] claim[s] 1 [to 22], wherein the pocket of the RRF protein is a pocket in the vicinity of C-terminal positioned on a folded part separating two domains of the RRF protein.

48.(amended) The method according to [any one of] claim[s] 1 [to 22], wherein the compound inhibits binding of the RRF protein to ribosome or inhibits behavior of the RRF protein on the ribosome.

49.(amended) The inhibitor to an RRF protein, obtained by the method according to [any one of] claim[s] 19 [to 23, 47 and 48].

9/pats

CRYSTAL OF RIBOSOMAL RECYCLING FACTOR (RRF) PROTEIN AND APPLICATION THEREOF ON THE BASIS OF THREE-DIMENSIONAL STRUCTURAL DATA OBTAINED FROM THE CRYSTAL

[TECHNICAL FIELD]

The present invention relates to a crystal of ribosomal recycling factor (hereinafter referred to as RRF). The present invention also relates to the steric structure of the RRF protein obtained by X-ray diffraction of the crystal. Further, the present invention relates to the determination of the structure of RRF mutants, homologues and so forth and technology for the development of bactericides, fungicides and herbicides of next generation by application of the RRF protein on the basis of the structural data and mechanism action of the RRF protein.

[BACKGROUND ART]

Protein biosynthesis is a function indispensable for the biological activities of all cells and consists of four steps, i.e., "initiation", "extension", "termination" and "ribosome recycling". The final step in the protein biosynthesis (the fourth step) terminates with the release and dissociation of a termination complex composed of messenger RNA, transfer RNA and ribosome individually in order for the ribosome to be recycled in the next "initiation" step. In Escherichia coli, which is a procaryote, it is known that

the ribosomal "recycling" is catalyzed by a ribosome recycling factor (hereinafter referred to as RRF), and an elongation factor G (hereinafter referred to as EFG), or a release factor 3. The process of the ribosomal "recycling" is introduced in the general remarks by Dr. Janosi et al. (1996 Adv. Biophys. 32:121-201) and the general remarks by Kaji et al. (Biochem, Biophys., Res Communs., 250 1-4, Protein, Nucleic Acid and Enzyme, Vol. 44, No. 7, 83-84 (1999)).

Since the possibility that in eucaryotes, the dissociation of a protein translation termination complex is catalyzed by a factor other than RRF is suggested and since the mRNA of eucaryotes is monocistronic whereas that of procaryotes is polycistronic (Kozak 1987, Mol. Cell Biol. 7:3438-3445; Das et al 1984, Nucleic Acids Res. 12:4757-4768; Schoner et al. 1986 Proc. Natl. Acad. Sci. U.S.A. 83:8506-8510; Sprengel et al. 1985 Nucleic Acids Res. 13:893-909), in eucaryotes, even if the dissociation of ribosome from mRNA is inhibited, this does not influence the downstream cistron. Thus, the fourth step, that is, the dissociation of protein translation termination complex, that corresponds to the final step of protein biosynthesis in eucaryotes is considered to be different from that in procaryotes and hence there is an expectation as a target of especially a new type of antibiotic.

On the other hand, presently a large number of antibacterial agents have been developed and among them there are those that exhibit very high bactericidal effect. However, the antibacterial agents

thus obtained include a lot of whose site of action remain unclear. Thus far, the development has centered on a method in which those antibacterial agents that exhibit activity are used as a material for random screening and while establishing a structure-activity relationship the one having a further utility is being developed. However, this requires an immensity of time and labor.

Accordingly, in recent years, formation of database is being attempted with a view to eliminating this problem and efficiently finding an inhibitor. On the basis of it, a rational drug design method is being studied and developed. An example of this includes an inhibitor of protease, which is an anti-HIV agent, recently put into market. The protease of HIV has been crystallized and its protein structure has been known. On the basis of the structure and three-dimensional structural amino acid sequence of the active site, one having the highest affinity for this site has been selected from known compounds by use of a computer and its inhibitory activity has been measured. By making a eutectic between the one having activity and the target protein and performing measurement of a three-dimensional structure, an anticipation of a compound that binds better can be made and hence, this is synthesized and its inhibitory activity is measured. Then, by making a eutectic between this substance and the target protein again and repeating the above-mentioned process, an extremely effective substance can be obtained.

Note that a large number of strains that have acquired a resistance to the conventional antibiotics as described above have been reported and a quick development of a novel antibiotic targeting the site that can directly control the growth of bacteria is needed. Accordingly, the present inventors have paid attention to the possibility that the above-mentioned RRF can be a new target of bactericides and advanced their study extensively. This idea has been being in the limelight.

[DISCLOSURE OF THE INVENTION]

Concerning RRF, the present inventors have thus far identified several kinds of gene sequences with regards to beginning with Escherichia coli, not only procaryotes but also eucaryotes (Japanese Patent Application Laid-open No. Hei 3-200797, PCT/JP98/00734, Japanese Patent Application No. Hei 10-150493). Therefore, up to secondary structures can be presumed from the amino acid sequences obtained therefrom. However, in the present state of the art, it has not reached that the actual protein structure can be identified from the secondary structures. In an actual protein, respective amino acid residues mutually act and undergo various modifications as the case may be to form its protein structure. Therefore, once the steric structure of a protein is discovered, it is possible to create a substance that can serve as a ligand thereof and in this sense, in order to create a useful antibiotic, the determination

of three-dimensional structure by crystallization will have an extremely important significance.

Therefore, an object of the present invention is to clarify the steric structure of RRF and contribute to the development of various antibacterial agents, antifungal agents and herbicides.

[BRIEF DESCRIPTION OF THE DRAWINGS]

[Fig. 1] is a photograph showing an XRRF protein crystal.

[Fig. 2] is a photograph showing an X-ray diffraction pattern of an XRRF protein crystal.

Details of diffraction pattern: xf1 to 1200 of 1200, yf 1 to 1200 of 1200

Direction of diffraction pattern: xf to the right, yf up

File order of data: -xf + yf

Maximum pixel value: 65535

Limit of scale: minimum = 1, maximum = 1,200, black indicates high value of diffraction intensity.

[Fig. 3] is a photograph showing of rendering by a ribbon of RRF. As shown in the drawing, it consists of two domains, one consisting of three helices and the second domain being a complex of a β-sheet and a coil helix.

[Fig. 4] is a photograph of a space-packing model of RRF.

[Fig. 5] is a schematic explanatory drawing showing a hypothesis on the function mechanism of RRF.

[Fig. 6] is a graph showing that the release of transfer RNA from a termination complex is inhibited by various inhibitors. The error bar indicates standard deviation.

[Fig. 7] is a graph by Lineweaver-Burk plot showing inhibition of ribosome release in the presence of a varied concentration of transfer RNA.

[Fig. 8] is a graph showing binding of RRF to ribosome is inhibited in the presence of paromomycicin. The error bar indicates standard deviation.

[DESCRIPTION OF REFERENCE NUMERALS]

1···Ribosome, 2···transfer RNA, 3···messenger RNA, 4···RRF, 5···EFG, 6···termination complex.

In consideration of the present status as described above, the present inventors have advanced study on RRF, during which they have been successful in obtaining a crystal of RRF and identifying its steric structure for the first time. They have further advanced the study and, as a result, completed the present invention.

Therefore, the present invention relates to an RRF protein crystal, a method of preparing thereof and a steric structure.

More specifically, the present invention relates to the method for designing a compound capable of binding to an active site or an accessory binding site of an RRF protein which comprises computationally evaluating a chemical entity of the RRF protein

on the basis of a structure coordinate obtained from an RRF protein crystal.

Also, the present invention relates to the method in which the RRF protein crystal is any crystal of the RRF protein itself, an RRF protein mutant, an RRF protein homologue or an RRF protein co-complex.

Also, the present invention relates to the method in which the RRF protein crystal is bipyramidal.

Further, the present invention relates to the method in which the RRF protein crystal has a space group $P4_12_12_1$ or a space group $P4_32_12$.

Also, the present invention relates to the method in which the RRF protein crystal has a size of 0.3 × 0.3 × 0.5 mm.

Also, the present invention relates to the method in which the RRF protein crystal has respective unit lattices of a size of a=b=47.3Å and c=297.6Å.

Further, the present invention relates to the method in which the RRF protein crystal is characterized by a structure coordinate described in Table 7.

Also, the present invention relates to the method in which the RRF protein crystal is derived from Thermotoga Maritima.

Also, the present invention relates to the method in which the RRF protein crystal is orthorhombic.

Also, the present invention relates to the method in which

the RRF protein crystal has a space group $P2_12_12$.

Also, the present invention relates to the method in which the RRF protein crystal has a size of 30 × 50 × 250 μm.

Also, the present invention relates to the method in which the RRF protein crystal is derived from strain X.

Further, the present invention relates to the method in which the RRF protein crystal is crystallized by a drop-like vapour diffusion method.

Also, the present invention relates to the method in which the RRF protein crystal is a heavy atom derivative and the crystal is any crystal of the RRF protein itself, an RRF protein mutant, an RRF protein homologue or an RRF protein co-complex.

Also, the present invention relates to the method in which the heavy atom derivative is formed by reaction of a compound selected from the group consisting of thyromethal, gold thiomalate, uranyl acetate and lead chloride.

Further, the present invention relates to the method in which the RRF protein crystal is a heavy atom derivative of platinum or mercury.

Also, the present invention relates to the method in which the RRF protein is a monomer.

Also, the present invention relates to the method in which the RRF protein is characterized by amino acid displacement according to Table 5 or Table 6.

Further, the present invention relates to the method in which a compound characterized by the chemical entity bound to the active site, accessory binding site or pocket is an inhibitor to the RRF protein.

Also, the present invention relates to the method in which the inhibitor is a competitive inhibitor, an uncompetitive inhibitor or a noncompetitive inhibitor to the RRF.

Also, the present invention relates to the method comprising determining orientation of a ligand at the active site or accessory binding site of the RRF protein.

Further, the present invention relates to the method in which the structure coordinate is a structure coordinate of the RRF protein according to Table 7.

Also, the present invention relates to the method in which the pocket of the RRF protein is a pocket in the vicinity of C-terminal positioned on a folded part separating two domains of the RRF protein.

Further, the present invention relates to the above-mentioned method in which the above-mentioned compound inhibits binding of the RRF protein to ribosome or inhibits the behavior of the RRF protein on ribosome.

The present invention also relates to an inhibitor of the RRF protein obtained by the above-mentioned method.

Also, the present invention relates to a method for searching a compound that can inhibit the activity of the RRF protein based

on the activity of inhibiting the binding of the RRF protein to ribosome or the activity of inhibiting the behavior of the RRF protein on ribosome.

Further, the present invention relates to an inhibitor of the RRF protein obtained by the above-mentioned method.

Also, the present invention relates to a method for determining the three-dimensional structure of RRF protein, including the steps of clarifying the crystal form of mutants, homologues or co-complexes of the RRF protein by molecular replacement.

The present invention also relates to an RRF protein crystal which is orthorhombic.

Also, the present invention relates to the RRF protein crystal having a space group $P2_12_12$ and size of 30 × 50 × 250 μm.

Also, the present invention relates to the RRF protein crystal in which the RRF is derived from strain X.

Also, the present invention relates to the RRF protein crystal which is bipyramidal.

Further, the present invention relates to the RRF protein crystal in which the RRF protein crystal has a space group $P4_12_12_1$ or a space group $P4_32_12$.

Also, the present invention relates to the RRF protein crystal having a size of 0.3 × 0.3 × 0.5 mm.

Also, the present invention relates to the RRF protein crystal having respective unit lattices of a size of a=b=47.3Å and c=297.6Å.

Also, the present invention relates to the RRF protein crystal characterized by amino acid displacement according to Table 5 or Table 6.

Further, the present invention relates to the RRF protein crystal characterized by a structure coordinate according to Table 7.

Also, the present invention relates to the RRF protein crystal derived from Thermotoga Maritima.

Also, the present invention relates to the RRF protein crystal crystallized by a drop-like vapour diffusion method.

Further, the present invention relates to the RRF protein crystal in which the crystal is any crystal of the RRF protein itself, an RRF protein mutant, an RRF protein homologue or an RRF protein co-complex.

Also, the present invention relates to an RRF protein in which amino acid in an active site is selected from the group consisting of Arg 110, Arg 129 and Arg 132 of SEQ. ID. NO. 1.

Also, the present invention relates to the RRF protein in which at least one amino acid in the active site or accessory active site is replaced by at least one amino acid selected from the group consisting of naturally occurring amino acids, non-natural amino acids, selenocysteine and selenomethionine.

Further, the present invention relates to the RRF protein in which a hydrophilic amino acid or a hydrophobic amino acid in the

active site or accessory active site is replaced.

Also, the present invention relates to the RRF protein in which at least one cysteine amino acid is replaced by an amino acid selected from the group consisting of selenocysteine and selenomethionine.

Also, the present invention relates to the RRF protein in which at least one methionine amino acid is replaced by an amino acid selected from the group consisting of selenocysteine or selenomethionine.

Further, the present invention relates to the RRF protein in which the RRF protein is in a crystal form.

Also, the present invention relates to the RRF protein having a specific activity higher or lower than that of a wild type enzyme.

Also, the present invention relates to the RRF protein having a varied substrate specificity.

Further, the present invention relates to use of the RRF protein for measuring binding interaction between a compound and the RRF protein.

Further, the present invention relates to the RRF protein in which at lest one amino acid residue on a surface of the RRF protein, in the surface or in the vicinity thereof is replaced and a change in surface charge by one or more charge units occurs.

Now that presently it is presumed that RRF is an ideal target of antibacterial agents, the three-dimensional structure of RRF clarified by the present invention is extremely important in the

industry since it directly associated with the development of antibacterial agents and the like. In addition, in consideration of the fact that the primary structures of RRFs of many pathogens are very close to each other (for example, the RRF of Pseudomonas aeruginosa has 60% homology to that of Escherichia coli), also concerning the three-dimensional structure of RRF of other pathogens, its clarification becomes extremely easy by the data of the three-dimensional structure of the RRF according to the present invention. Therefore, also in order to develop species-specific antibacterial agents, the present invention is extremely useful in the development of next generation antibiotics, fungicides and dezymotizing agents on the basis of RRF inhibition, in particular as an index upon developing antibacterial agents by rational drug design.

The terms used in the present specification are defined as follows.

"RRF protein" means an RRF protein having an enzyme activity in an ordinary state.

"Naturally occurring amino acid" means an L-isomer of a naturally occurring amino acid. The naturally occurring amino acids are glycine, alanine, valine, leucine, isoleucine, serine, methionine, threonine, phenylalanine, tyrosine, tryptophane, cysteine, proline, histidine, aspartic acid, asparagine, glutamic acid, glutamine, γ-carboxyglutamic acid, arginine, ornithine and

lysine. Unless specifically indicated, amino acids in the present specification are L-forms.

"Non-natural amino acid" means an amino acid that is not naturally found in proteins. As examples of the non-natural amino acid used in the present specification, there can be cited racemi mixtures of selenocysteine and selenometionine. Further, the non-natural amino acid includes D- or L-forms of norleucine, para-nitrophenylalanine, homophenylalanine, para-fluorophenylalanine, 3-amino-2-benzylpropyionic acid, and homoarginine, and D-phenylalanine.

"Positively charged amino acid" includes any optional naturally occurring amino acid or non-natural amino acids having a positively charged side chain under normal physiological conditions. As examples of the positively charged naturally occurring amino acid, there can be cited arginine, lysine and histidine.

"Negatively charged amino acid" includes any optional naturally occurring amino acid or non-natural amino acids having a negatively charged side chain under normal physiological conditions. As examples of the negatively charged naturally occurring amino acid, there can be cited aspartic acid and glutamic acid.

"Hydrophobic amino acid" means any optional amino acid that has a relatively water-insoluble, uncharged and nonpolar side chain.

Examples of the naturally occurring hydrophobic amino acid are alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophane and methionine.

"Hydrophilic amino acid" means any optional amino acid that has a relatively water-soluble, uncharged and polar side chain. Examples of the naturally occurring hydrophilic amino acid are serine, threonine, tyrosine, asparagine, glutamine, and cysteine.

"Mutant" refers to an RRF polypeptide characterized by substitution of at least one amino acid in the RRF sequence of wild type E. coli (that is, polypeptide showing the biological activity of wild type RRF). Such a mutant can be obtained by the expression of cDNA of RRF in which mutation has occurred in the sequence encoded thereby, for example, by oligonucleotide specific induction. The RRF mutant can be obtained by a general biosynthetic method by Noren, C.J. et al. (Science, 224, p182-188 (1989)) by site specific incorporation of non-natural amino acids to the RRF protein.

Selenocysteine or selenomethionine is incorporated by wild type or mutant type RRF by the expression of cDNA encoding the RRF in auxotrophic E. coli strain. In this method, the wild type or mutant type RRF cDNA does not contain one (or both) of natural cysteine and natural methionine and can be expressed in a host on a growth medium enriched with selenocysteine or selenomethionine (or both). Further, selenomethionine can be incorporated into a wild type or mutant type RRF by a methionine metabolism inhibiting method (Van

Dyne, G.D. et al., J. M. B., 229 pp105 (1993)).

"Change in surface charge" means a change in at least one charge unit of a mutant polypeptide at a physiological pH in comparison with the wild type RRF. This can be obtained by mutation of at least one amino acid of the wild type RRF into an amino acid having a side chain having a charge different from that of the wild type side chain at a physiological pH. The change in surface charge can be determined by measuring the isoelectric point of the polypeptide having a substituted amino acid and comparing it with the isoelectric point of the wild type RRF molecule.

"Change in substrate specificity" refers to a change in substrate of the mutant RRF in comparison with the wild type RRF. The substrate specificity (species specificity) is determined by separating ribosome, tRNA and EF-G from a pathogen and based on whether or not they can be used as substrates for RRF of E. coli and RRF mutants.

"Kinetic form" refers to the state of an enzyme in a free form or in a non-bound form, or the state of an enzyme bound to a chemical entity either at its active site or an accessory active site.

"Competitive" inhibitor is an inhibitor that inhibits the RRF activity by binding to the same kinetic form of RRF as that to which the substrate of RRF binds, and therefore, by directly competing with the active site of RRF.

"Uncompetitive" inhibitor is an inhibitor that inhibits RRF

by binding to a different kinetic form of RRF from that to which the substrate binds.

"Noncompetitive" inhibitor is an inhibitor that binds to either the free form or substrate-bound form of RRF.

"Homologue" means a protein that has at least 30% amino acid sequence homology with RRF or any optional function domain of RRF.

"Co-complex" means RRF, mutant or homologue of RRF bound to a chemical entity or compound through a covalent bond or non-covalent bond.

"β-Sheet" refers to a conformation of polypeptide chain extending in an expanded zigzag conformation. Polypeptide chain portions extending in parallel all extend in the same direction. The polypeptide chains extending in anti-parallel extend in the direction opposite to the direction of the parallel lines.

"Active site" or "active site portion" refers to any optional site or all sites in RRF as below. The substrate-binding site is a site to which ribosome and its complex binds and at which decomposition of the substrate occurs. The active site is in the vicinity of at least amino acid residue 110, 129, and 132 by use of SEQ. ID. No. 1.

"Structure coordinate" refers to a mathematical coordinate obtained according to a formula relating to the pattern obtained by the diffraction of X-ray monochromatic bean by an atom (dispersion center) of RRF molecule in the form of crystal. The dispersion data

is used for calculating an electron density map of the repeating unit of crystal and the electron density map is used for establishing the positions of respective atoms in the unit lattice of crystal.

"Heavy atom derivative" refers to a chemically modified form of RRF protein crystal. In making it, in actuality, the crystal is dipped in a solution containing a heavy metal atom salt or an organometal compound that can diffuse through the crystal and bind to the surface of a protein (for example, lead chloride, gold thiomaleate, thyromethal or uranyl acetate). The position or positions of bound heavy metal atom or atoms can be determined by X-ray diffraction analysis of the dipped crystal. Then, using the data, phase data used for constructing the three-dimensional structure of an enzyme are prepared. One skilled in the art will understand that the set of structure coordinate determined by X-ray crystallography has a standard error. For the purpose of the present invention, any set of structure coordinates of RRF, RRF homologue or RRF mutant having a root-mean-square value deviation of protein backbone atoms (N, Cα, C and 0) of less than 0.75Å when superposed on the structure coordinates enumerated in Table 7 should be considered the same.

"Unit lattice" refers to a block of a fundamental parallelohexahedron. Total volume of the crystal can be constructed by repeated regular stacking of such blocks.

"Space group" refers to an arrangement of objective elements

of a crystal.

"Molecular substitution" refers to a method including a step of orienting and positioning another molecule whose structure coordinates (for example, the structure coordinates shown in Table 7) are known in the unit lattice of an unknown crystal to thereby prepare a provisional model of RRF crystal whose structure coordinates are unknown such that the observed diffractive pattern of the unknown crystal can be optimally explained. Then, a phase is calculated based on this model and synthesized with the observed amplitude to obtain an approximate Fourier synthesis of the structure whose coordinates are unknown. Then, application to a purified substance enables one to finally obtain an accurate structure of the unknown crystal. By use of the structure coordinates of RRF of the present invention and by use of molecular substitution, the structure coordinates of a mutant, homologue, or co-complex or different crystal structure of RRF can be determined.

In examples of the present invention, crystallization and structural analyses were performed using strain X derived RRF and Thermotoga Maritima derived RRF. However, practice can also be made similarly on other RRFs. Also, upon crystallization, not only RRF protein itself but also, RRF protein mutants, RRF protein homologues, and RRF protein co-complexes can be crystallized and the respective structures can be analyzed.

"Pocket" refers to a hollow that is present on an RRF protein

surface and also includes in addition to a binding pocket that is present on a binding site or accessory binding site of an RRF protein, other pockets that do not participate in binding to a substrate and so forth upon the expression of the activity of RRF.

The present invention provides for the first time the crystals of RRF of strain X and of Thermotoga Maritima RRF and the structures of RRF determined therefrom. On the other hand, the crystal of Thermotoga Maritima RRF was formed from an ammonium sulfate solution. The crystal has space group $P4_32_12$ of a bipyramid type. The unit lattice of the crystal has a=b=47.3Å, c=297.6Å. The structure coordinate of RRF is shown in Table 7. The crystal packing indicates that RRF is a monomer.

Fig. 3 shows an illustration of Thermotoga Maritima RRF by a ribbon. Helices A, B, C, D, E, and F indicate helices that exist from N-terminal toward C-terminal. β-Sheets 1, 2, 3, 4, 5, and 6 are numbers of β-sheets that exist from N-terminal toward C-terminal. As shown in the figure, RRF consists of 2 domains, one consisting of 3 helices and the 2nd domain being a complex of β-sheet coil and a helix. The active site mounts on the E and F helices in the figure and in order to maintain the activity, it is important to retain the three-dimensional structure of the domain including the helices B, C, and D and β-sheets 1, 2, 3, 4, and 5.

Fig. 4 represents a space-packing model of Thermotoga Maritima RRF and N and C indicate N-terminal and c-terminal, respectively.

The gray color represents a carbon atom, red represents an oxygen atom, violet represents an N atom. The number shows the SEQ. ID. Number of amino acid sequence and 1 designates N-terminal.

Thus, based on the data concerning the three-dimensional structure of RRF elucidated by the present inventors, the identification of the active site and accessory binding site of an enzyme has been made possible for the first time. It has revealed together with the results of RRF gene mutation described later that there is a high possibility that the active site portion contains at least amino acid residues Arg 110, Arg 129 and Arg 132 of SEQ. ID. NO. 1.

The present invention for the first time makes it possible to use a molecular design technology in which chemical entity and compound are designed, selected and synthesized with respect to RRF. The chemical entity and compound include inhibitory compounds that can bind to all or a portion of the active site or accessory-binding site of RRF. Upon approaches made possible by the present invention, a compound binding to an enzyme is designed and the structure coordinate of RRF is used in order to modify the physical properties (for example, solubility) of the compound in various methods. For example, the present invention makes it possible to design a compound that acts as a competitive inhibitor of RRF by binding all or a portion of the active sites of RRF. The present invention also makes it possible to design a compound that

acts as an uncompetitive inhibitor of RRF. These inhibitors can bind to all or a portion of the accessory binding sites of the RRF that has already bound to the substrate and can be more potent than the competitive inhibitor that binds only to the active site of RRF and more nonspecific. Similarly, noncompetitive inhibitors that inhibit RRF by binding thereto can be designed by use of structure coordinate obtained by the present invention regardless of whether it is bound or not bound to another chemical entity.

A second design approach is to confirm an RRF crystal based on molecules composed of various chemical bodies in order to determine an optimal site for the interaction between an RRF inhibitor candidate and RRF. For example, high resolution X-ray diffraction data recovered from a crystal that is saturated with a solvent makes it possible to determine the position of solvent molecules of each type. Then, a small molecule that strongly binds to these sites can be designed and synthesized and tests on its inhibitory activity can be performed (Travis, J., Science, 262, p1374 (1993)).

The present invention is useful in designing improved analogue of an RRF inhibitor or designing a novel class of inhibitors based on reaction intermediate of RRF and RRF inhibitor co-complex in the reaction of other compound that binds to substrate or RRF with the RRF. This provides a novel means for designing an RRF inhibitor having both high specificity and high stability.

Another approach that is made possible and facilitated by the

present invention is to perform screening by use of a computer on a chemical entity or compound that can bind to RRF entirely or partially. In this screening, the property of conformity of such a chemical entity or compound to the binding site can be judged by either shape complementarity or estimated interaction energy (Meng, E.C. et al. J. Comp. Chem., 13, 505-524 (1992)).

In the case where RRF can be crystallized in more than one crystal form, structure coordinate or a part of it of RRF as provided by the present invention is particularly important for the analysis of the structure of other crystal form of RRF. The structure coordinate or a part of it of RRF can also be used for the analysis of the structure of an RRF mutant, the structure of an RRF co-complex, or the structure of the crystal form of any other optional protein having a significantly homologous amino acid sequence to any optional functional domain of RRF.

One of the methods that can be used for this purpose is molecular substitution. In this method, whether or not an unknown crystal structure is the crystal form of another form of RRF, RRF mutant or RRF co-complex or any other optional protein having an amino acid sequence that is significantly homologous to any optional functional domain of RRF can be determined by use of the structure coordinate of RRF of the present invention as provided in Table 7. This method provides accurate structural form about an unknown crystal more quickly and efficiently than trying to determine such

data from the beginning.

Furthermore, according to the present invention, RRF mutants can be crystallized by way of its co-complexes with known RRF inhibitors. Then, a series of crystal structures of such complexes can be analyzed by molecular substitution and compared with the crystal structure of wild type RRF. Therefore, a promising site for modification among various binding sites of the enzyme can be identified. Based on the data, a means for determining the most effective binding interaction (for example, an increased hydrophobic interaction) between RRF and the chemical entity or compound is provided.

All the above-mentioned complexes can be studied by use of a known X-ray diffraction technology and made precise so as to have an R value of about 0.20 or less in comparison with 2 to 3 Å resolution X-ray data by use of a computer software (for example, X-POLAR, Yale University, 1992, distributed by Molecular Simulation, Inc)(for example, Blundel & Johnson, Protein Crystallography, Academic Press (1967), Methods in Enzymology, Vol. 114, 115, H.W. Wycoff et al., Academic Press (1985)). Therefore, the data can be used for optimizing an RRF inhibitor and more importantly, can be used for designing a novel RRF inhibitor and synthesize it. The structure coordinate of RRF provided in the present invention facilitates the identification of related protein, enzyme or nucleic acid similar to RRF in function, structure or both of them. This enables more

proper presumption of active site, binding site and so forth of RRF itself and the above-mentioned similar protein and the like, which leads to a novel antibacterial agents, herbicide or fungicide.

In designing a compound that binds to or inhibits the RRF of the present invention, generally two elements must be considered. First, the compound must physically and structurally bind to the RRF. As the non-covalent bond intermolecular interaction important for the binding of RRF to its substrate, there can be cited hydrogen bond, van der Waals force and hydrophobic interaction.

Secondly, the compound must be one on which a conformation that enables binding to the RRF can be supposed. A specified part of a compound may not directly participate in binding to the RRF but the part can still influence the whole conformation of the molecule. This also gives a considerable influence on the effectiveness. As the necessary conditions of such a conformation, there can be cited three-dimensional structure and orientation as a whole of a chemical entity or compound relating to all or a part of binding sites (for example, active site or accessory binding site of RRF), or distance between functional groups of a compound containing some chemical bodies that directly interact with the RRF.

Potential inhibitory effect or binding effect of a chemical compound on the RRF can be analyzed before the compound is actually synthesized and tested by use of a computer modeling technology. In the case where a theoretical structure of a predetermined compound

suggests the existence of insufficient interaction and binding between the compound and the RRF, the synthesis and test of the compound can be avoided. However, in the case where the computer modeling suggests strong interaction, the molecule can be synthesized and its inhibitory capability can be tested by the method of Hirashima and Kaji (Biochemistry, 11, 4037 (1972)) or a method using an oligonucleotide, and in vivo screening (Japanese Patent Application No. Hei 10-158643). By this method, the synthesis of compounds that are ineffective can be avoided.

The inhibitory compounds of the RRF or other bound compounds of the RRF can be evaluated by a computer and chemical bodies or fragments can be designed by a means of a series of steps of screening and selecting their ability of binding to respective binding pockets or other regions of the RRF.

There may be used one of methods of screening the chemical bodies or fragments on their ability of binding to respective binding pockets of RRF, more particularly the binding site or accessory binding site of the RRF, or to other pockets not participating in binding to the substrate or the like upon expression of the activity of the RRF. This process can be started by visual study of the active site, for example, upon computer screening based on the RRF coordinate in Table 7. For example, as shown in the sketch by a ribbon in Fig. 3, the RRF that has a form of "L"-shape has a pocket in the vicinity of C-terminal positioned at the "L"-shaped bent portion that

separates the two domains. A compound bound to the pocket can be a leading candidate of an inhibitory compound to the RRF. The above-mentioned pocket can be readily observed by preparing a space-packing model based on the RRF coordinate shown in Table 7 by use of a software of Rasmol et al. The pocket is positioned between the two domains of the RRF and hence, it suggested the possibility that it participates in the activity of the RRF through adjustment of the angle between the domains. Then, the selected fragment or chemical entity can be positioned in various orientations or can couple to the respective binding pockets of the RRF. The coupling can be achieved by use of software such as Quanta and Sybyl and thereafter, minimization of energy and molecular kinetics are performed by use of a standard molecular mechanism force field (for example, CHARMM, AMBER).

A specialized computer program can aid the process of selecting a fragment or chemical entity. Examples of the programs include the followings:

The following program is available from Oxford University, oxford, UK: GPID (Goodford, P. J., "A Computational Procedure for Determining Energetically Favorable Binding Sites on Biologically Important Macromolecules", J. Med. Chem., 28, pp. 849-857 (1985)), and the following is available from Molecular Simulations, Burlington, MA: MCSS (Miranker, A or M. Karplus "Functionality Map of Binding Sites: A Multiple Copy Simultaneous Search Method.",

Proteins: Structure, Function and Genetics, 11, pp. 29-34 (1991)). As for AUTODOCK (Goodsell, D. S. and A. J. Olsen, "Automated Docking of of Substrates to Proteins by Simulated Annealing", Proteins: Structure Function and Genetics, 8 pp. 195-202, it is available from Scripps Research Institute La Jolla, CA, and as for DOCK (Kuntz, I, D. et al, "A Geometric Approach to Macromolecule-Ligand Interactions", J. Mol. Biol., 161, pp 269-288 (1982)), it is available from University of California, San Francisco, CA.

Once a proper chemical entity or fragment is selected, the chemical entity or fragment can be assembled into a single compound or inhibitor. The assembling can be performed by way of visual study of interrelationship fragments in a three-dimensional image displayed on a computer screen with respect to the structure coordinate of the RRF. Then, model construction is performed by a manual by use of software such as Quanta or Sybyl.

As examples of useful programs that can help one skilled in the art in the case where respective chemical bodies or fragments are contacted, there can be cited the followings:

The following program is available from the University of California, Berkeley, CA: CAVEAT (Bartlett, P. A. et al, "CAVEAT: A Program to Facilitate the Structure-Derived Design of Biologically Active Molecules", Molecular Recognition in Chemical and Biological Problems", Royal Chem, Soc., 78, pp. 182-196 (1989)). A region of 3D Database systems such as MACCS-3D (MDL Information Systems, San

Diego, CA is generally explained in Martin, Y. C., "3D database Searching in Drug Design", J. Med. Chem., 35, pp. 2145-2154 (1992). As for HOOK, it is available from Molecular Simulations, Burlington, MA.

Instead of constructing an RRF inhibitor from one fragment or chemical entity at a time in a stepwise manner as described above, the inhibitory compound or other RRF bound compound can be designed wholly or anew by use of the active site (or one containing some parts of a known inhibitor as needed) from the RRF. As these methods there can be cited the followings:

The following program is available from Biosym Technologies, San Diego, CA: LUDI (Bohm, H. J., "The Computer Program LUDI: "A New Method for the de novo Design of Enzyme Inhibitors", J. Comp, Aid, Molec, Design, 6 pp. 61-78(1992). As for LEGEND (Nishibata, Y. and A. Itai, tetrahedron, 47, p.8985 (1991), it is available from Molecular Simulations Burlington, MA. As for LeapFrog, it is available from Tripos Associates, St. Louis, MO.

Other molecular modeling can be used in the present invention. For example, the following should be referred: Molecular Modeling Software and Methods for Medicinal Chemistry, J. Med. Chem., 33, p883-894 (1990) by Cohen, N. C., et al. or Navia, M. A. and M. A. Murcko, The Use of Structural Information in Drug Design, Current Opinions in Structural Biology, 2, p. 202-210(1992).

Once a compound is designed or selected by the above-mentioned

method, the effectiveness in that the compound can bind to the RRF can be tested by computer evaluation and then optimized. For example, for a compound that is designed or selected so as to function as an RRF inhibitor, preferably a volume must be studied that does not overlap the volume occupied by the active site in the case where it binds to a natural substrate. An effective RRF inhibitor must preferably show a relatively small difference in the energy between its bound state and free state (that is, in a small binding stress). Therefore, the most effective RRF inhibitor must be designed with a binding stress of not exceeding about 10 kcal/mol, preferably about 7 kcal/mol. The RRF inhibitor can interact with an enzyme in a similar conformation having an overall binding energy of more than 1. In such cases, the stress of binding is in a difference between the energy of the free compound and the average energy of conformations observed in the case where the inhibitor binds to the enzyme.

The compound designed or selected such that it binds to the RRF can be optimized by use of a computer in such a manner that preferably it does not have a repulsive electrostatic interaction with the target enzyme. As such a noncomplementary (for example, static charge) interaction, there can be cited repulsive charge-charge interaction, dipole-dipole interaction and charge-dipole interaction. Specifically, totaling all the electrostatic interaction between the inhibitor when it is bound

to the RRF and the enzyme, neutral or preferable contribution is made to the enthalpy of bond.

Specified computer software for evaluating the stress and electrostatic interaction of a compound can be utilized in this field of art. As examples of program designed for such uses, there can be cited Gaussian 92 C, M. J. Frisch, Gaussian, Inc., Pittsburgh, PA 1992; AMBER, version 4.0 P. A. Kollman, University of California, San Francisco, 1994; QUANTA/CHARMM Molecular Simulations, Inc., San Diego, CA 1994 and so forth. These programs can be practiced by use of a general computer such as Silicon Graphics IRIS 4d/35 or IBM RISC/6000 Model 1550 or the like. Other hardware and software are known to one skilled in the art.

Once an RRF bound compound is optimally selected or designed as described above, then substitution of some of atoms or side chains of the compound is performed in order to improve or modify the binding properties thereof. Generally, first substitution is conservative. That is, the substituent has substantially the same size, shape, hydrophobicity and charge as the original group. Those compounds of which it is known to change conformation in the art must be avoided. The chemical compounds thus substituted are analyzed of their effectiveness for conformity with the RRF in the same manner as the method by use of a computer as described above.

The present invention also makes it possible to make mutants of the RRF and elucidate their crystal structure. More specifically,

the present invention makes it possible to determine a desired site for mutation by the active site, accessory binding site and position of interface of the RRF on the basis of the crystal structure of the RRF.

For example, mutation may be directed to a specified site or combination out of sites of wild type RRF, that is, active site or accessory binding site only. Alternatively, for the induction of mutation, the position on the interface site is selected. Similarly, only the position on or near the enzyme surface may be substituted to generate a change in surface charge by 1 or more charge unit in comparison with the wild type enzyme. Alternatively, the amino acid residue of the RRF may be selected on the basis of its hydrophilic or hydrophobic property.

Such mutants are characterized by any of the different properties as compared with the wild type RRF. For example, such mutants may have a change in surface charge by 1 or more charge units or an increased stability to dissociation of subunits. Alternatively, such mutants may have a change in substrate specificity as compared with the wild type RRF or specific activity higher or lower than that of the wild type RRF.

The RRF mutant that is prepared by the present invention can be prepared by many methods. For example, the wild type RRF sequence can be mutated at a site that has been identified to be desirable for mutation by utilizing the present invention by use of an

oligonucleotide-specific mutation induction or other conventional technical (for example, depletion or the like) means. Alternatively, mutants of RRF can be made by site-specific substitution of a specified amino acid by an amino aid that does not naturally occur. Furthermore, the RRF mutants can be made by substitution of a specified cysteine or methionine residue by selenocysteine or selenomethionine. This is achieved by growing a host organism that can express either a wild type polypeptide or a mutant polypeptide on a growth medium that does not contain natural methionine or cysteine (or both) but is enriched with selenocysteine or selenomethionine (or both).

Mutation can be introduced in a DNA sequence encoding the RRF by use of synthetic oligonucleotides. The oligonucleotides include nucleotide sequence adjacent to a desired mutation site. Mutation can be made in a sequence of the full-length DNA sequence of RRF, RRF of other organism or shortened or elongated (deleted or added) RRF sequences.

According to the present invention, mutant RRF DNA sequences made by the above-mentioned method or substitute methods known in the art can be expressed by use of an expression vector. As is well known in the art, the expression vector contains an element that is typically independent of the host genome and enables self replication in the host cell and one or more phenotype markers for the purpose of selection. The expression vector also contains before or after an insert of a DNA sequence surrounding a desired RRF mutant

code sequence, a promoter, an operator, a ribosome-binding site, and a translation-starting signal and optionally a control sequence encoding a repressor gene and a stop signal. In some embodiments, in the case where secretion of produced mutant is desired, the nucleotide encoding the signal sequence may be inserted before the RRF mutant encoding sequence. For the expression under control of the control sequence, a desired DNA sequence must be operatively coupled to the control sequence. That is, it must have a suitable start signal before a DNA sequence maintaining a suitable leading frame that encodes the RRF mutant and enables the expression of this sequence under control by the control sequence and production of a desired product encoded by the RRF sequence.

A wide variety of well-known expression vectors that can be utilized are useful for expressing the mutated RRF encoding sequence of the present invention. These include vectors composed of segments of chromosomal DNA sequences, non-chromosomal DNA sequences and synthetic DNA sequences, such as, for example, various known derivatives of SV40, known bacteria plasmids (for example, E. coli derived plasmids such as col E1, pCR1, pBR322, pMB9 and derivatives thereof), plasmids of a wider host spectrum (for example, RP4), phage DNA (for example, many derivatives of λ phage (for example, NM989) and other DNA phages (for example, M13 and filamentous single strand DNA phage)), yeast plasmids such as 2μ plasmid or its derivatives, and vectors obtained by combination of plasmid and

phage DNA (for example, plasmid modified in order to utilize phage DNA or other expression control sequences). In a preferred embodiment of the present invention, the present inventors utilize E. coli vector.

Furthermore, any of a wide variety of expression control sequences that controls the expression of a DNA sequence when operatively coupled to the DNA sequence is used in a vector in order to express the mutated DNA sequence of the present invention. As such a useful expression control sequence, there can be cited, for example, early promoter and later promoter of SV40 for animal cells, lac system, trp system, TAC system or TRC system, major operator region and promoter region (all for E. coli) of λ phage control region of fd coat protein, promoter of 3-phosphoglycerate kinase or other sugar decomposing enzyme, promoter of acid phosphatase (for example, Pho5), promoter of yeast α-mating factor for yeast, and other sequences known to control gene expression of prokaryote cell, eucaryote cell or virus and combinations thereof. In a preferred embodiment of the present invention, the present inventors utilize E. coli expression.

A wide variety of species of hosts are also useful for the production of mutant RRF according to the present invention. As such a host, there can be cited, for example, bacteria such as E. coli, Bacillus and Streptomyces, fungi such as yeast, animal cells such as CHO cell and COS-1 cell, plant cells and transgenic host

cells. In a preferred embodiment of the present invention, the host cell is E. coli.

It should be understood that when all the expression vectors and expression systems express the mutant DNA sequence of the present invention and produce modified RRF or RRF mutants, they do not always function in the same manner. Not all the hosts use the same expression system to equally function well. However, one skilled in the art can make selection from these vectors, expression control sequences and hosts without performing experiments or departing from the scope of the present invention. For example, the matter that is important when selecting vectors is the ability of a vector to replicate in a predetermined host. The number of copy of vector, ability of controlling the number of copy, and expression of other proteins to be encoded by the vector such as an antibiotic marker must also be taken into consideration.

Upon selecting the expression control sequence, various factors must be taken into consideration. These are, for example, relative strength of the system, its controlling power, conformity of DNA sequence encoding the modified RRF of the present invention, in particular conformity with respect to potential secondary structure.

The host must be selected in consideration of conformity with the selected vector, the toxicity of the modified RRF to the host, the ability of secreting a mature product, the ability of properly

folding the protein, fermentation requirement, easiness of purification of the modified RRF from the host, and safety. Among these parameters, one skilled in the art can select various vector/expression control system/host combinations that can produce a useful amount of mutant RRF.

The mutant RRF produced in these systems can be purified various conventional steps and strategies including steps and strategies used for purifying the wild type RRF.

Once RRF mutation is made at a desired position (that is, active site or accessory binding site), the mutant can be tested on any one of some objective properties.

For example, the mutant may be screened on a change in charge at a physiological pH. This can be determined by the isoelectric point of the mutant RRF in comparison with the isoelectric point (pI) of the wild type parent. The isoelectric point can be measured by gel electrophoresis by the method of Wellner, D., Analyt. Chem., 43, p597 (1971). The mutant in which surface charge has changed is an RRF polypeptide having a substituted amino acid positioned at a surface of the enzyme and a changed pI as provided by the structural data of the present invention.

Furthermore, the mutant may be screened with respect to specific activity that is high or low as compared with the wild type RRF. The mutant is measured of its activity by use of the method of Hirashima and Kaji and the assay by use of oligonucleotide (cited

above). The mutant can be tested on a change in RRF substrate specificity by measuring the RRF reaction as described above.

Furthermore, an object of the present invention includes a mutant having increased safety. The RRF mutant having increased safety includes one that exhibits no loss of enzyme activity.

Hereinafter, the present invention will be illustrated in detail by examples. The examples shown below are merely for the purpose of detailed explanation thereof but by no means are meant to exclude other methods.

[EXAMPLES]

Example 1. Crystallization of RRF protein of strain X by drop-like vapour diffusion technique

5 μl of a solution containing 4 mg/ml to 8 mg/ml of RRF protein of strain X, 50 mM Tris hydrochloride, pH 8.5, 70-100 mM of sulfuric acid salt and 14% to 18% of polyethylene glycol was converted into droplets and equilibrated in a liquid pool containing a crystallizing reagent having a higher concentration than that of the droplets. The equilibration was performed until the vapour pressure of droplets became identical to the vapor pressure of the liquid pool due to diffusion of the volatile medium (water or organic solvent). When the equilibration occurred due to water exchange (from the droplets to the liquid pool) the volume of the droplets was changed. As a result, the concentrations of all the media in the droplets changed.

In case of the medium having a higher vapour pressure than that of water, exchange from the liquid pool to the droplets occurred. In the instant example, the glass vessel that the RRF protein solution contacted was used after its surface was subjected to hydrophobic treatment. By dialysis against a buffer composed of 100 mM of Tris hydrochloride, pH 8.5, 150 mM to 200 mM of sulfuric acid salt, 28% to 36% of polyethylene glycol, XRRF crystal was obtained. The crystal grew to a size of 30 × 50 × 250 μm over 1 to 3 weeks. The results are shown in Fig. 1.

Example 2. Three-dimensional structure of RRF by X-ray diffractive analysis

As a means for determining the RRF three-dimensional structure, a multiple isomorphous replacement procedure was used. This is a standard method necessary for obtaining diffusion data of a heavy atom from isomorphous protein crystal. A difference between the unreplaced one and the isomorph was calculated from the positions of the heavy atom to form a Patterson map. In preparing a protein model, the data on initial protein phase necessary for the calculation of electron density diagram were calculated by use of several kinds of derivatives.

The X-ray diffraction data of the refrigerated crystal were collected in BL71 by MaxII synchrotron (Sweden, Lund).

The native crystal was diffracted at a resolution of 2.6Å.

Because of the problem of mosaicity of 1.5 or more, a resolution of 2.9Å or less was thus far used. This typical diffraction pattern is shown in Fig. 2.

The native data analysis was completed and Rsym was found 1.0. The statistical data are shown in Fig. 1.

The crystal has a=98.5Å, b=106.7Å, and c=66.7Å and belongs to $P2_12_12$.

The asymmetric unit includes 2 to 4 molecules and there are translations of 0.5, 0.33 and 0.5 between the molecules. Data of two derivatives were obtained and the derivative of platinum diffracted at 4.0Å and the derivative of mercury diffracted at 3.8Å.

Statistical study of native data

Table showing summary of diffractive intensity and R-factor indicated in terms of the size of shell (resolution)

Value of R (as a linear function) = 3D SUM (ABS(I-<I>))/SUM (I)

Value of R (as a quadratic function) = 3D SUM ((I-<I>)**2) / SUM (I**2)

χ^2 = 3D SUM ((I-<I>)**2) / (error **2*N/(N-1)

For all calculations of addition, calculation was performed only on values measured twice or more.

Table 1

Lower limit and upper limit of shell Angstrom		Diffraction intensity		Average	Normal	Linear multiplier	Quadratic multiplier
		Average	Error	Stat. value	$\chi 2$ value	R-factor	R-factor
Lower limit	Upper limit						
30.0	7.12	814.6	36.6	18.5	0.709	0.033	0.032
7.12	5.67	227.1	16.7	13.5	1.012	0.084	0.072
5.67	4.95	266.0	19.0	15.3	0.983	0.083	0.075
4.95	4.50	417.3	25.3	18.4	1.052	0.070	0.064
4.50	4.18	394.0	25.5	19.6	1.239	0.087	0.086
4.18	3.93	330.4	24.4	19.7	1.064	0.093	0.088
3.93	3.74	296.1	24.6	20.9	1.286	0.125	0.219
3.74	3. 58	283.8	25.5	22.0	1.275	0.142	0.221
3.58	3.44	207.5	23.2	21.2	1.314	0.170	0.192
3.44	3.32	173.2	22.5	21.1	1.278	0.193	0.298
3.32	3.22	151.8	22.1	20.9	1.414	0.222	0.231
3.22	3.12	130.4	21.7	20.8	1.560	0.265	0.280
3.12	3.01	108.3	20.6	19.9	1.552	0.306	0.307
3.04	2.97	92.2	19.9	19.2	1.655	0.334	0.315
2.97	2.90	74.9	19.2	18.7	1.632	0.411	0.416
Total reflection		268.4	23.2	19.3	1.259	0.119	0.102

Example 3. Crystal structure of RRF of Thermotoga Maritima

RRF cDNA of Thermotoga Maritima was cloned in an expression vector (PET1650) and expressed in E. coli by addition of IPTG. As a result, Thermotoga Maritima RRF accumulated in the host cells at a high level. The cells were mechanically destroyed and purified

by a method which is modified from the method of Hirashima and Kaji (Biochemistry, 11, 4037, (1972)) to obtain Thermotoga Maritima RRF. The crystal of RRF was grown by vapour diffusion. Mixing 5-10 μl of the RRF solution with the same amount of reservoir liquid (00.1 M of sodium acetate, 2.0 M of ammonium sulfate (pH 5.5), 5 mM of DTT, and 10% of glycerol), equilibrating the mixture with 600 μl of the above-mentioned reservoir liquid at 25°C, and streaking this liquid for 24 hours to accelerate crystal formation resulted in the appearance of crystal after 15 hours. Then, after 3 days, this grew into a crystal of bipyramid type of 0.3 × 0.3 × 0.5 mm.

It is possible for one skilled in the art to properly modify the above-described crystallization conditions.

All the X-ray dataset (2.55Å resolution) were collected in a Mar 345 Image Plate Detector by use of MAXII synchrotron in BEAMLINE BL711. Merge, scale, indexing and integration of data were performed by use of programs of VDS and Xscale (Kabsch, W. J. Appl. Crystallography 26 795 (1993)). MAD data (multiwarelength anomalous dispersion data) were performed in BEAMLINE BM14 ESRF at a wavelength of 0.9184 to 0.978 and 0.9788 Å and collected by use of a Mar 345 Image Plate Detector. The data were processed by use of Mosflm (Leslie, A. G. W. in Crystallographic computing oxford Univ Press (1990)), and scale and merge were performed in Scala (CCP4).

For the position of selenium atom in the RRF, shelx program

(sheldrick, G. M. Acta Cryst. A46 P467 (1998)) was used and a normalized structural factor was used. A heavy atom (selenium) parameter was made precise by use of MIphase (CCP4). Trial made to obtain electron density maps based on both space groups $P4_12_12$ and $P4_32_12$ revealed that correct space group was $P4_32_12$. An average merit value indicated a resolution of 0.66Å to 4.0Å. Table 2 shows the crystal data of Thermotoga Maritima RRF.

Table 2

Data collection

The highest resolution shell, 2.65 - 2.55 Å

Resolution	30-2.55
Total observations	79947 (7119)
Unique reflections	11927 (1261)
Average redundancy	6.7 (5.6)
R_{sym} (%)	0.049 (0.156)
Data completeness (%)	99.8 (99.1)
I/sigma(I)	26.4 (8.9)

$R_{merge} = (\sum|I-\langle I\rangle|/\sum I)$ where I is the observed intensity and $\langle I\rangle$ is the average intensity of symmetry-related reflections.

Values in parentheses refer to the values at maximum resolution.

The map was improved by solvent flattering and phase expansion (CCD4-collaborative conpurity project #4, A suite of program for protein crystallography Daiesburg Laboratory, Warrington, WA4 4. AD UK (1979) and it was successful in tracing a complete polypeptide chain. Precision ((CNS) ranger A. T. et al., Acta Cryst D54 p905 (1998)) of the position of model construction program (Jones, A.T. et al., Acta Cryst. A47 PP110 (1991)) and phase combination were

repeated to thereby enable all the side chains to be introduced into the model. Rigid body and positional precision were performed and further simulated annealing was carried out. This model was compared with the crystal data (native data) to reach a final model only by use of the phase of the model.

The model of the RRF of the present invention has an R factor (25.3%) for all the observed data on X- and Y-axes. Root-mean-square value deviation from ideal bond length and bond angle are 0.01Å and 2.0Å, respectively.

Table 3 shows results of statistical processing of Thermotoga Maritima RRF crystal data.

Table 3

Data collection statistics

Dataset (wavelength Å)	Peak (0.9786)	Inflection (0.9788)	Remote (0.9184)
Resolution (Å)	2.9	2.9	2.9
Completeness (%)	99	98	98
Rsym (%)	7.0	7.0	7.2
Cullis R-centric	0.67	0.56	-
Cullis R-anomalous	0.60	0.77	0.78

Table 4 shows phasing of Thermotoga Maritima RRF.

Table 4 shows Phasing of Thermotoga Maritima RRF

Table 4

Resolution

Phasing						
Resolution bin (Å)	30-11.5	11.5 - 8.1	8.1 - 6.2	6.2 - 5.1	5.1 - 4.3	4.3 - 3.7
FOM	0.704	0.732	0.745	0.683	0.623	0.58
Mean FOM	0.66					

2. Presumption of the active site of RRF

To presume the position of active site in the RRF molecule, a series of RRF mutants were produced. Mutation induction was performed by use of a PCR method that involved many errors to introduce mutation (Janosi et al., EMBO J. 17 1141 (1998)).

Isolation of a plasmid having frr (gene encoding RRF) having a lethal gene mutation was performed as follows. PMIX described in Janosi et al., EMBO J. 17 1141 (1998) was used. To explain briefly, pMIX is a plasmid obtained by causing various gene mutations to occur in frr and introducing the frr into a chloramphenicol-resistant plasmid. In this example, Escherichia coli LJ4 (recA-) was used as a host. Since this bacterium has on chromosome frr inactivated by frame shift, so that the Escherichia coli keeps life by means of wild type frr on pPEN(1560) (Janosi et al., EMBO J. 17 1141 (1998)). PPEN(1560) includes kanamycin resistant factor and sucrose sensitivity gene.

The Escherichia coli was transformed with pMIX and selected with chloramphenicol resistance as a marker. Since frr is indispensable to bacteria, the bacteria having a lethal mutation in pMIX cannot live without having the above-mentioned pPEN1560. Therefore, bacteria having both plasmids, pMIX and pPEN1560 were searched. Incidentally, the both plasmids, pMIX and pPEN1560 do not usually live together since they are incompatible. However, they live together if they are driven by necessity (necessity of antibiotic marker and frr) as described above.

To select such an Escherichia coli, the transformant was inoculated on a plate containing CM and sucrose, and further was replica plated on a plate containing CM and KM. Selecting a bacterium that grows in the latter but does not grow in the former enables selection of a bacterium that has pPEN1560 and lethal frr in pMIX. Since this bacterium has pPEN1560, it cannot grow on a plate containing sucrose. Out of 153 transformants thus obtained respective plasmids were purified with which Escherichia coli DH5α (having wild type frr) was transformed. Since this bacterium has wild type frr as described above, it does not require pPEN1560 (kanamycin resistance). Therefore, selecting chloramphenicol- and kanamycin-sensitive Escherichia coli DH5α enables selection of Escherichia coli having a lethal mutation and having pMIX.

From the thus obtained and Escherichia coli, plasmids were isolated and Kpn1-HindIII fragment (0.9 kb, frr) was taken out and

DNA sequence determination by a common method was performed. The results are shown in Table 5.

Table 5 *Genetic mutations inactivating RRF*†

Mutational class	Allele	Number of isolates	Nucleotide change	Change in the primary sequence of RRF	Notes
Single AA change	*frr146*	1	T(152)C	Leu(51)Pro	-
	frr160	1	T(161)C	Leu(54)Pro	-
	frr161	1	T(194)C	Leu(65)Pro	-
	frr109	1	T(99)C T(194)C	Leu(65)Pro	Ser(33)silent
	frr106	1	C(123)T G(329)A	Arg(110)His	Val(41)silent
	frr119	3	C(-91)A C(385)T	Arg(129)Cys	promoter involved
Class A	*frr114*	1	C(394)G	Arg(132)Gly	-
	frr132	1	C(394)T	Arg(132)Cys	-
	frr133	1	G(395)A	Arg(132)His	-
	frr138	1	G(395)A	**Arg(132)Gln**	-
	frr124	2	T(524)C	Leu(175)Pro	-
	frr149	2	C(447)T T(524)C	Leu(175)Pro	Ser(149)silent
Double AA change	*frr165*	1	G(28)A A(490)C	Glu(10)Lys Thr(164)Pro	-
	frr113	1	G(28)C G(162)A A(490)C	Glu(10)Lys Thr(164)Pro	Leu(154)silent
Class B	*frr112*	1	T(38)C T(512)C	Met(13)Thr Ile(171)Thr	-
	frr116	2	T(107)A C(317)A	Leu(36)Gln Thr(106)Lys	-
	frr118	1	A(269)G G(329)A	Asn(90)Ser Arg(110)His	-
Triple AA change	*frr134*	1	T(14)C A(103)G T(194)A	Ile(5)Thr Ser(35)Gly Leu(65)Glu	-
Class C	*frr141*	1	G(88)A T(97)C C(394)T	Gly(30)Ser Ser(33)Pro Arg(132)Cys	-
C-terminal truncation via early stop	*frr127*	1	G(52)T T(416)C	Glu(18)stop (17 AA long RRF)	+mutation beyond stop
	frr123	1	A(76)T	Lys(26)stop (25 AA long RRF)	-
	frr158	1	C(135)G	Tyr(45)stop (44 AA long RRF)	-
	frr125	1	C(157)T	Gln(53)stop (52 AA long RRF)	-
	frr162	1	T(24)C C(157)T	Gln(53)stop (52 AA long RRF)	Asp(8)silent
Class D	*frr140*	1	A(196)T T(206)C G(309)T	Lys(66)stop (65 AA long RRF)	+2 mutations beyond stop
	frr110	1	C(218)A	Ser(73)stop (72 AA long RRF)	-
	frr131	1	C(-6)A C(218)A	Ser(73)stop (72 AA long RRF)	SD-initiation spacer involved
	frr108	1	G(364)T A(540)G	Glu(122)stop (121 AA long RRF)	-
	frr117	1	A(430)T	Lys(144)stop (143 AA long RRF)	-

	frr136	1	T(336)C A(430)T	Lys(144)stop (143 AA long RRF)		Asp(112)silent
	frr159	1	T(-46)C A(508)T	Lys(170)stop (169 AA long RRF)		+mutation between promoter and SD
	frr142	3	G(514)T	Glu(172)stop (171 AA long RRF)	-	
C-terminal	*frr115*	2	A(103)G C(157)T	Ser(35)Gly	Glu(53)stop (52 AA long RRF)	-
truncation & single	*frr151*	1	(A11)G G(364)T	Asp(4)Gly	Glu(122)stop (121 AA long RRF)	-
AA change	*frr140*	1	A(61)G C(367)T	Lys(21)Glu	Glu(123)stop (122 AA long RRF)	-
	frr166	1	A(61)G G(162)A C(367)T	Lys(21)Glu	Gln(123)stop (122 AA long RRF)	Leu(154)silent
Class E	*frr121*	1	A(445)G C(469)T	Ser(149)Gly	Gln(157)stop (156 AA long RRF)	-
	frr152	1	C(467)T C(469)T	Ser(156)Phe	Gln(157)stop (156 AA long RRF)	-
	frr139	1	C(467)T C(469)T C(555)T	Ser(156)Phe	Gln(157)stop (156 AA long RRF)	mutation beyond stop
C-terminal	*frr170*	1	T(5)del	Stop at nt 50-52	(16 AA long RRF)	Ser(3) to (Cys(16) changed
truncation preceded	*frr101*	1	G(40)del	Stop at nt 50-52	(16 AA long RRF)	Asp(14) to Cys(16) changed
by frame-shift	*frr169*	1	A(70)del	Stop at nt 170-172	(56 AA long RRF)	Ile(24) to Ser(56) changed
	frr143	1	A(79)del	Stop at nt 170-172	(56 AA long RRF)	Ile(27) to Ser(56) changed
	frr129	1	C(75)del	Stop at nt 170-172	(56 AA long RRF)	Ser(25) to Ser(56) changed
	frr126	1	C(101)del A(419)T	Stop at nt 170-172	(56 AA long RRF)	Ser(35) to Ser(56) changed +mutation beyond stop
Class F	*frr105*	1	T(170)del A(359)T T(492)C	Stop at nt 176-178	(58 AA long RRF)	Val(57) to Thr(58) changed +2 mutations beyond stop
	frr147	1	CG(82-83)del	Stop at nt 210-212	(69 AA long RRF)	Arg(28) to Val(69) changed
	frr153	1	AT(199-200)C	Stop at nt 266-268	(88 AA long RRF)	Ile(67) to Gly(88) changed
	frr107	1	A(333)del	Stop at nt 338-340	(112 AA long RRF)	Asp(112) changed
	fr103	1	C(362)del	Stop at nt 416-418	(138 AA long RRF)	Ala(121) to Lys(138) changed
	frr137	1	A(346)del A(363)GA(501)G	Stop at nt 416-418	(138 AA long RRF)	Ile(116) to Lys(138) changed +mutation in shifted sequence +mutation beyond stop
	frr145	1	A(389)del	Stop at nt 416-418	(138 AA long RRF)	Asn(130) to Lys(138) changed
	frr135	1	A(511)del	Stop at nt 545-547	(181 AA long RRF)	Ile(168) to Glu(181) changed

†In description of nucleotide and amino acid changes, the positions of change are indicated by a number in parenthesis preceded by description of the wild-type nucleotide or amino acid and followed by mutated ones. The amino acids are described by their three letter codes. Abbreviations: AA=amino acid; del=deletion

As shown in this table, 61 strains were obtained. These had 53 different genotypes. It was confirmed that the thus separated plasmid having a lethal gene did not function as frr by use of LJ4 having temperature-sensitive RFF (Janosi et al., EMBO 17 1141 (1998)). All the obtained plasmids were not able to support the growth of LJ4 at 42°C.

Then, a method of separating amino acid mutation that does not influence the function of frr from the structural gene of frr will be described. For this purpose, LJ4 was used as a host. Since the frr on chromosome of this host does not function as described above, it can exist at 27°C by means of plasmid pKH6 having frr14.

Since this Escherichia coli lives by means of frr14 (that encodes temperature-sensitive RRF), naturally it is temperature-sensitive. When the Escherichia coli was grown at 27°C at a natural reversion rate, one that was grown at 42°C were obtained in a ratio of 4.2×10^{-6}. Of the Escherichia coli one that becomes temperature-sensitive again by replacing the plasmid with one having tsfrr(pKH6) was selected and the DNA sequence of the frr portion was determined by a common procedure. In all the frrs thus obtained, the gene mutation Val 117 Asp of frr returned to wild type valine. Several kinds of them showed mutation at amino acid sites other than the 117-position. The mutations showed no influence on the function of frr. The mutations are shown in Table 6.

Table 6

Genetic mutations reverting the temperature sensitivity of RRF†

Allele	Phenotype	Number of isolates	Nucleotide in position...					Amino acid in position...			Notes
			350	457	458	511	513	117	153	171	
frr	*wt*	NA	T	G	A	A	T	Val	Asp	Ile	{222}
frr14	ts	NA	A	G	A	A	T	Asp	Asp	Ile	{2785}
frr201	tr revertant	2	T	G	A	A	T	Val	Asp	Ile	-
frr206	tr revertant	3	T	T	A	T	T	Val	Tyr	Phe	-
frr204	tr revertant	1	T	T	A	T	A	Val	Tyr	Leu	-
frr202	tr revertant	3	T	G	A	T	A	Val	Asp	Leu	-
frr203	tr revertant	1	T	G	A	A	A	Val	Asp	Ile	Ile(171)silent
frr205	tr revertant	4	T	G	C	T	T	Val	Ala	Phe	-

†Amino acids are represented by their 3-letter codes. Abbreviations: NA=not applicable; wt=wild-type; ts=temperature senitive; tr=temperature resistant

3. Presumption of function mechanism of RRF

From the sketch by ribbon of RRF shown in Fig. 2, the space packing model shown in Fig. 3 and so forth, it revealed that the RRF has a shape and size similar to transfer RNA (Selmer, M., Al-Karadaghi, s., Hirokawa, G., Kaji, A. & Liljas, A., Science 286, 2349-2352 (1999)). Therefore, the possibility is suggested that the RRF expresses protein translation termination complex releasing activity by showing a behavior similar to that of transfer RNA. Specifically, a model as shown in Fig. 5 is suggested on the function mechanism of the RRF.

First, an RRF 4 binds to the aminoacyl site (A site) of a termination complex 6 (a) composed of transfer RNAs 2a, 2b, a messenger RNA 3 and a ribosome 1. To the RRF 4, an EFG 5 with GTP binds. Also, to the peptidyl site (P site) and exit site (E site) of the ribosome 1, the transfer RNAs 2a, 2b bind, respectively (b). Then, ribosome-dependent hydrolysis of GTP and translocation of RRF4 bound to the A site to the P site are caused by the EFG 5. At the same time, release of the transfer RNA 2b bound to the E site and release of the transfer RNA 2a bound to the P site via the movement to the E site take place and finally two molecules of transfer RNA are released (c). Finally, subsequent to the release of the RRF 4 and of the EFG 5 from the ribosome 1, the ribosome 1 is released from the messenger RNA 3 to complete the dissociation of the termination complex 6 (d).

To verify the hypothesis on the basis of this model, the following experiments were performed.

Example 4. Inhibition of the RRF activity by aminoglycosides

Aminoglycosides such as streptomycin, paromomycin, and gentamycin are known to inhibit themselves from binding to the A site of the transfer RNA by binding to the A site of ribosome (Moazed, D. & Noller, H. F., Nature 327, 389-394 (1987); Fourmy, D., Yosizawa, S. & Publisi, J. D., J. Mol. Biol., 277, 333-345 (1988)); Yoshizawa, S., Fourmy, D. & Publisi, J. D., EMBO J., 17, 6437-6448 (1988)). Therefore, according to the model in Fig. 5, the above-mentioned aminoglycosides should inhibit the binding of RRF to the A site and if such a binding is inhibited, the dissociation process of termination complex due to the RRF should also be inhibited. Accordingly, whether the dissociation process of termination complex is inhibited or not in the presence of the above-mentioned aminoglycosides was examined by use of the amount of transfer RNA released from the ribosome and the amount of ribosome released from the messenger RNA as indices.

The release of deacylating transfer RNA from ribosome was examined by the following method.

Polysome (Hirashima, A. & Kaji, A., J. Mol. Biol., 65, 43-58 (1972)) obtained from E. coli Q13 strain treated with tetracycline (0.6 to 1.8 A_{260} unit) was incubated in 550 µl of buffer R (10 mM Tris-Cl, pH 7.4, 8.2 mM magnesium sulfate, 80 mM ammonium chloride,

and 0.14 mM ditiothritol (DTT)) in the presence of 275 μM of puromycin, 0.2 nmole of RRF, 0.2 nmole of EFG (Kaziro, Y., Inoue-Yokosawa, N. & Kawakita, M., E. coli J. Biochem. 72, 853-863 (1972)) and 0.37 mM of GTP at 30°C for 15 minutes. Then, as the aminoglycosides, streptomycin, paromomycin or gentamycin was added in an amount of 200 μM, 100 μM or 100 μM, respectively. Then, by centrifugation at 330 G for 40 minutes by use of Microcon 100 (produced by Millipore, trade name), the released transfer RNA was separated from the termination complex. Then, to the above-mentioned Microcon 100, 550 μl of buffer J (10 mM Tris-Cl, pH 7.6, 10 mM magnesium sulfate, 50 mM ammonium chloride, and 0.5 mM DTT) was poured and the mixture was centrifuged to wash the filter once. The combination of the washing solution and filtrate was centrifuged at 14,000 G for 15 minutes twice by use of Microcon 30 (produced by Millipore, trade name) to concentrate it to 14 μl. Then, the concentrated transfer RNA was aminoacylated with 0.15 μCi of ^{14}C-amino acid mixture (produced by Amersham, 52 mCi per 1 mg of carbon atom) dissolved in 30 μl of a buffer solution (50 mM Tris-Cl, pH 7.8, 10 mM magnesium acetate, 6 mM β-mercaptoethanol, 3 mM ATP, 5 mM phosphoenolpyruvic acid, 138 μg of pyruvate kinase, 33.3 μg of aminoacyl transfer RNA synthetase (Monmose, K. & Kaji, A., Arch. Biochem. Biophys., 111, 245-252 (1965)). The thus obtained radioactivity that is insoluble in cold trichloroacetic acid (4°C) corresponds to ^{14}C-aminoacyl transfer RNA and the amount thereof was calculated on the basis of the

radioactivity of a known amount of transfer RNA labeled by the same method. The aminoglycosides used are produced by Sigma Co. Also, the termination complex was obtained from natural polysome of E. coli treated with puromycin (Hirashima, A. & Kaji, A., J. Biol. Chem., 248, 7580-7587 (1973)). It has been known that each ribosome in the isolated polysome in the stage of after completion of translocation and generally bear two molecules of transfer RNA (Remme, J., Margus, T., Villems, R. & Nierhaus, K. H. Eur. J. Biochem. 183, 281-284 (1989)); Stark, H. et al., Cell 88, 19-28 (1977)).

The results are shown in Fig. 6. In Fig. 6, positive control is a value obtained when RRF, EFG and GTP were added without adding aminoglycosides while negative control is a value obtained when none of aminoglycosides, RRF, EFG and GTP was added.

The release of ribosome from messenger RNA was examined by the following method.

The above-mentioned polysome (0.5 to 0.8 A_{260} unit) was incubated in 270 μl of buffer R in the presence of 275 μM of puromycin, 1 nmole of purified RRF (Hirashima, A. & Kaji, A., Biolchemistry, 11, 4037-4044 (1972)), 1 nmole of EFG^{32}, 0.37 mM of GTP and aminoglycosides at 30°C for 15 minutes. As the aminoglycosides, streptomycin, paromomycin or gentamycin was added in an amount of 100 μM, 5 μM or 5 μM, respectively. Then on 5 ml of sucrose with a density gradient of 15 to 30% in the buffer J, the above-mentioned incubated buffer solution was stacked and centrifuged under the

conditions of 40,000 rpm for 75 minutes at 4°C by use of Beckman SW50.1. The absorbance at 254 nm was monitored by an ISCO UA-6 detector and the concentration of free 70S ribosome was measured. The results are shown in Table 7. The values in Table 7 were expressed in percentage of the concentration of free 70S ribosome in the presence of aminoglycosides to the concentration of free 70s ribosome in the case where no glycoside was added (control). In control, about 42% of total ribosome (occupying approximately 90% of polysome) was converted into monosome by the RRF.

Table 7 Release of ribosome from mRNA by RRF and EF-G in the presence of various inhibitors

Inhibitor	Concentration	Release of ribosome from mRNA by RRF (percentage to control)
Control	-	100*
Paromomycin	5μm	16.5
Gentamycin	5μm	0
Streptomycin	100μm	9.9
Thiostreptone	20μm	0
Viomycin	50μm	0
Fusiginic acid	200μm	0
GMPPCP	370μm****	0

From Fig. 6, it can be seen that in the presence of the aminoglycosides, the release of transfer RNA was inhibited to a value identical to or less than that of the negative control. Also, from Table 7, it can be seen that in the presence of the aminoglycosides, the release of ribosome was also inhibited. These results strongly suggest that coupling of the RRF to the A site of ribosome is necessary for the expression of the activity of RRF. Therefore, it can be considered that the substance that inhibits binding of the A site of the RRF can be a potent candidate of an RRF inhibitor.

Example 5. Inhibition of RRF activity by thiostreptone and viomycin

Thiostreptone and viomycin are both EFG inhibitors and are known to inhibit the translocation of transfer RNA (Pestka, S., Biochem. Biophys. Res. Commun. 40, 667-674 (1970); Rondnina, M. V., Savelsbergh, A., Katunin, V. I. & Wintermeyer, W. Nature 385, 37-41 (1997); Rodnina, M. V., et al., Proc. Natl. Acad. Sci. USA 96, 9586-9590 (1999)). Therefore, according to the model in Fig. 5, thiostreptone and viomycin must inhibit the translocation of the RRF bound to the A site to the P site. If so, the release of transfer RNA from the P site and E site that must have occurred along with the translocation and further the process of release of the termination complex by the RRF must be inhibited. Accordingly, whether or not the dissociation process of termination complex is inhibited or not in the presence of thiostreptone or viomycin was examined by use of the amount of transfer RNA released from ribosome and the amount of ribosome released from messenger RNA as indices.

The release of transfer RNA from ribosome was examined in the same manner as in Example 4 except that thiostreptone (produced by Sigma Co.) or viomycin (produced by ICN Co.) was added in place of the amidoglycosides. Thiostreptomycin or viomycin was added in an amount of 100 μM or 200 μM, respectively. The results are shown in Fig. 6.

Also, the release of ribosome from messenger RNA was examined in the same manner as in Example 4 except that thiostreptone or

viomycin was added in place of the amidoglycosides and that in the case where thiostreptone was added, DMSO was added to 0.06% in reaction compounds. Thiostreptomycin or viomycin was added in an amount of 20 μM or 50 μM, respectively. The results are shown in Table 7.

From Fig. 6, it can be seen that in the presence of thiostreptone or viomycin, the release of transfer RNA was inhibited to a value identical to that of the negative control. Also, from Table 7, it can be seen that in the presence of thiostreptone or viomycin, the release of ribosome was completely inhibited. These results strongly suggest that the translocation of the RRF bound to the A site to the P site is necessary for the expression of the activity of RRF. Therefore, it can be considered that the substance that inhibits the translocation of the RRF can be a potent candidate of an RRF inhibitor.

Example 6. Inhibition of termination complex release by GMPPCP and fusidic acid

It has been known that GMPPCP and fusidic acid are both EFG inhibitors and inhibits the release of termination complex by fixing EFG to ribosome after the translocation of transfer RNA while allow the occurrence of translocation of transfer RNA only once (Inoue-Yokosawa, N., Ishikawa, C. & Kaziro, Y., J. Biol. Chem. 249, 4321-4323 (1974); Rodnina, M. V., Savelsbergh, A., Katunin, V. I. & Wintermeyer, W., Nature, 385, 37-41 (1997); Bodley, J. W., Zieve,

F. J., Lin, L., & Zieve, S. T., J. Biol. Chem., 245, 5656-5661 (1970); Kuriki, Y., Inoue, N. & Karizo, Y., Biochim. Biophys. Acta, 224, 487-497 (1970)). Therefore, according to the model in Fig. 5, GMPPCP and fusidic acid allow the translocation of the RRF bound to the A site to the P site, so that they must not inhibit the releasse of transfer RNA from the P site and E site that takes place along with the translocation. On the other hand, since GMPPCP and fusidic acid inhibit the release of termination complex, they must inhibit the release of ribosome from messenger RNA. Accordingly, whether or not the release of transfer RNA from ribosome and release of ribosome from messenger RNA are inhibited or not in the presence of GMPPCP or fusidic acid was examined.

The release of transfer RNA from ribosome was examined in the same manner as in Example 4 except that GMPPCP or fusidic acid was added in place of the amidoglycosides and that in the case where GMPPCP was added, the experiment was performed in the absence of GTP. GMPPCP or fusidic acid was added in an amount of 370 μM or 200 μM, respectively. The results are shown in Fig. 6.

Also, the release of ribosome from messenger RNA was examined in the same manner as in Example 4 except that GMPPCP (produced by Sigma Co.) or fusidic acid (produced by ICN Co.) was added in place of the amidoglycosides and that in the case where GMPPCP was added, the experiment was performed in the absence of GTP. GMPPCP or fusidic acid was added in an amount of 370 μM or 200 μM, respectively.

The results are shown in Table 7.

From Fig. 6, it can be seen that even when GMPPCP or fusidic acid was added, the release of transfer RNA was at a value identical to that of the positive control, so that the release of transfer RNA is not inhibited. On the other hand, from Table 7, it can be seen that GMPPCP or fusidic acid completely inhibits the release of ribosome (Igarashi, K., Ishitsuka, H. & Kaji, A., Biochim. Biophys. Res. Commun., 37, 499-504 (1969); Hirashima, A. & Kaji, A., J. Mol. Biol., 65, 43-58 (1972); Ogawa, K. & Kaji, A., Eur. J. Biochem., 58, 411-419 (1975); Karimi, R., Pavlov, M. Y., Buckingham, R. H. & Ehrenberg, M., Molecular cell, 3, 601-609 (1999)).

These results more strongly suggest the possibility that the RRF expresses termination complex release activity by showing a similar behavior to that of transfer RNA and furthermore, the fact that the release of transfer RNA and the release of ribosome can be separately inhibited supports the model shown in Fig. 5 in which the release of termination complex proceeds stepwise by the EFG and RRF.

Example 7. Inhibition of RRF activity by the presence of excessive deacylating transfer RNA

It was strongly suggested by example 4 that it is necessary that the RRF binds to the A site of ribosome for the expression of the activity of the RRF. Accordingly, if excess of transfer RNA

exists, RRF and transfer RNA competitively bind to the A site of ribosome and as a result the dissociation of termination complex will must be inhibited. To confirm this point, inhibition of dissociation of termination complex in the presence of varied concentration of transfer RNA was examined.

Polysome (0.5 to 1 A_{260} unit) was incubated in the presence of varied amounts between 0 and 1,000 pmole of RRF, EFG, and GTP, and a varied amount between 0 and 10 nmole of transfer RNA. Then, after performing sucrose density gradient centrifugation (density gradient: 15 to 30%), absorbance at 254 nm was measured to thereby measure the amount of free ribosome. The graph by Lineweavwe-Burk plot prepared on the basis of the measurement results is shown in Fig. 7. The vertical axis represents a reciprocal of the percentage of the amount of free ribosome in the presence of a varied amount of transfer RNA to the amount of free ribosome in the absence of transfer RNA.

In the graph in Fig. 7, the vertical intercept is constant regardless of the amount of transfer RNA and K_m increases as the amount of transfer RNA increases, so that it can be seen that transfer RNA competitively inhibits the activity of RRF.

Example 8. Inhibition by paromomycin of binding of RRF to ribosome

On the basis of the results in Example 4 and Example 7, the following experiments were performed in order to directly confirm

that paromomycin used in Example 4 inhibits binding of RRF to ribosome.

First, less than 1 pmole of ^{35}S labeled histidine tagged RRF (^{35}S-His-RRF) was incubated in a buffer solution (50 mM Tris-Cl, pH 7.6, 10 mM magnesium acetate, 30 mM potassium chloride, and 1 mM DTT) in the presence of 10 pmole of washed ribosome and a varied concentration of paromomycin at 30 °C for 10 minutes. After removing free ^{35}S-His-RRF by microconcentration, the amount of ribosome-bound ^{35}S-His-RRF was measured by radioactivity. The ^{35}S-His-RRF was prepared by purifying in vitro His-RRF expressed by in the presence of ^{35}S-labeled methionine by use of Ni^{2+} beads. The results are shown in Fig. 8. The binding ratio of RRF is a value calculated by taking the amount of RRF bound to ribosome in the absence of paromomycin as 100%.

From Fig. 8, it can be seen that the binding ratio of RRF decreases depending on the concentration of paromomycin. Therefore, paromomycin is considered to inhibit the activity of RRF by inhibiting the binding of the RRF to ribosome.

Table 8

Structure cooedinates of RRF

ATOM	Type	Residue	#	X	Y	Z	OCC	B
ATOM	1	CB VAL	2	10.355	24.444	73.500	1.00	50.36
ATOM	2	CG1 VAL	2	11.185	25.300	72.669	1.00	50.36
ATOM	3	CG2 VAL	2	9.102	25.267	74.125	1.00	50.36
ATOM	4	C VAL	2	8.502	23.777	72.304	1.00	83.11
ATOM	5	O VAL	2	8.267	24.906	71.890	1.00	83.11
ATOM	6	N VAL	2	10.415	23.206	71.242	1.00	83.11
ATOM	7	CA VAL	2	9.881	23.282	72.625	1.00	83.11
ATOM	8	N ASN	3	7.567	22.901	72.348	1.00	72.42
ATOM	9	CA ASN	3	6.364	23.543	72.193	1.00	72.42
ATOM	10	CB ASN	3	5.458	22.851	71.191	1.00	77.31
ATOM	11	CG ASN	3	4.541	23.842	70.630	1.00	77.31
ATOM	12	OD1 ASN	3	3.368	23.859	70.934	1.00	77.31
ATOM	13	ND2 ASN	3	5.109	24.802	69.910	1.00	77.31
ATOM	14	C ASN	3	6.085	23.280	73.622	1.00	72.42
ATOM	15	O ASN	3	6.652	22.357	74.186	1.00	72.42
ATOM	16	N PRO	4	5.407	24.244	74.267	1.00	41.97
ATOM	17	CD PRO	4	4.846	25.501	73.729	1.00	61.56
ATOM	18	CA PRO	4	5.121	24.010	75.681	1.00	41.97
ATOM	19	CB PRO	4	3.981	24.999	75.973	1.00	61.56
ATOM	20	CG PRO	4	3.931	25.973	74.808	1.00	61.56
ATOM	21	C PRO	4	4.632	22.521	75.634	1.00	41.97

ATOM	22	O	PRO	4	4.781	21.774	76.617	1.00 41.97
ATOM	23	N	PHE	5	4.080	22.114	74.491	1.00 37.43
ATOM	24	CA	PHE	5	3.593	20.751	74.270	1.00 37.43
ATOM	25	CB	PHE	5	2.898	20.648	72.912	1.00 36.55
ATOM	26	CG	PHE	5	1.608	21.414	72.832	1.00 36.55
ATOM	27	CD1	PHE	5	1.285	22.160	71.693	1.00 36.55
ATOM	28	CD2	PHE	5	0.697	21.370	73.868	1.00 36.55
ATOM	29	CE1	PHE	5	0.081	22.866	71.612	1.00 36.55
ATOM	30	CE2	PHE	5	-0.505	22.072	73.792	1.00 36.55
ATOM	31	CZ	PHE	5	-0.815	22.815	72.652	1.00 36.55
ATOM	32	C	PHE	5	4.720	19.724	74.322	1.00 37.43
ATOM	33	O	PHE	5	4.603	18.687	74.974	1.00 37.43
ATOM	34	N	ILE	6	5.812	19.998	73.620	1.00 41.88
ATOM	35	CA	ILE	6	6.946	19.073	73.616	1.00 41.88
ATOM	36	CB	ILE	6	7.997	19.477	72.561	1.00 41.38
ATOM	37	CG2	ILE	6	9.276	18.658	72.740	1.00 41.38
ATOM	38	CG1	ILE	6	7.410	19.266	71.162	1.00 41.38
ATOM	39	CD1	ILE	6	8.209	19.943	70.059	1.00 41.38
ATOM	40	C	ILE	6	7.585	19.045	74.997	1.00 41.88
ATOM	41	O	ILE	6	7.926	17.982	75.516	1.00 41.88
ATOM	42	N	LYS	7	7.738	20.222	75.592	1.00 47.17
ATOM	43	CA	LYS	7	8.309	20.333	76.923	1.00 47.17
ATOM	44	CB	LYS	7	8.293	21.792	77.371	1.00 68.01
ATOM	45	CG	LYS	7	8.925	22.024	78.727	1.00 68.01

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ATOM     46  CD  LYS     7       8.706  23.444  79.215  1.00 68.01
ATOM     47  CE  LYS     7       9.525  23.709  80.466  1.00 68.01
ATOM     48  NZ  LYS     7       8.752  24.480  81.480  1.00 68.01
ATOM     49  C   LYS     7       7.451  19.496  77.872  1.00 47.17
ATOM     50  O   LYS     7       7.963  18.740  78.701  1.00 47.17
ATOM     51  N   GLU     8       6.137  19.639  77.732  1.00 39.77
ATOM     52  CA  GLU     8       5.175  18.920  78.555  1.00 39.77
ATOM     53  CB  GLU     8       3.758  19.392  78.230  1.00 50.17
ATOM     54  CG  GLU     8       2.678  18.705  79.047  1.00 50.17
ATOM     55  CD  GLU     8       1.291  18.857  78.443  1.00 50.17
ATOM     56  OE1 GLU     8       0.319  18.370  79.058  1.00 50.17
ATOM     57  OE2 GLU     8       1.170  19.456  77.351  1.00 50.17
ATOM     58  C   GLU     8       5.269  17.406  78.349  1.00 39.77
ATOM     59  O   GLU     8       5.227  16.637  79.311  1.00 39.77
ATOM     60  N   ALA     9       5.385  16.979  77.095  1.00 39.35
ATOM     61  CA  ALA     9       5.487  15.558  76.790  1.00 39.35
ATOM     62  CB  ALA     9       5.452  15.336  75.294  1.00 32.04
ATOM     63  C   ALA     9       6.764  14.965  77.375  1.00 39.35
ATOM     64  O   ALA     9       6.751  13.859  77.905  1.00 39.35
ATOM     65  N   LYS    10       7.872  15.690  77.285  1.00 37.72
ATOM     66  CA  LYS    10       9.120  15.182  77.840  1.00 37.72
ATOM     67  CB  LYS    10      10.277  16.113  77.514  1.00 61.11
ATOM     68  CG  LYS    10      10.990  15.829  76.231  1.00 61.11
ATOM     69  CD  LYS    10      12.171  16.770  76.124  1.00 61.11
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ATOM	70	CE	LYS	10	12.574	17.020	74.678	1.00	61.11
ATOM	71	NZ	LYS	10	13.323	18.307	74.569	1.00	61.11
ATOM	72	C	LYS	10	9.037	15.051	79.358	1.00	37.72
ATOM	73	O	LYS	10	9.508	14.073	79.939	1.00	37.72
ATOM	74	N	GLU	11	8.462	16.064	79.996	1.00	33.88
ATOM	75	CA	GLU	11	8.310	16.076	81.445	1.00	33.88
ATOM	76	CB	GLU	11	7.601	17.353	81.879	1.00	67.13
ATOM	77	CG	GLU	11	8.505	18.337	82.587	1.00	67.13
ATOM	78	CD	GLU	11	8.166	19.783	82.266	1.00	67.13
ATOM	79	OE1	GLU	11	6.969	20.147	82.296	1.00	67.13
ATOM	80	OE2	GLU	11	9.107	20.558	81.989	1.00	67.13
ATOM	81	C	GLU	11	7.529	14.860	81.929	1.00	33.88
ATOM	82	O	GLU	11	7.989	14.119	82.792	1.00	33.88
ATOM	83	N	LYS	12	6.349	14.648	81.365	1.00	39.27
ATOM	84	CA	LYS	12	5.528	13.518	81.766	1.00	39.27
ATOM	85	CB	LYS	12	4.137	13.637	81.143	1.00	39.81
ATOM	86	CG	LYS	12	3.424	14.898	81.559	1.00	39.81
ATOM	87	CD	LYS	12	2.028	14.985	80.996	1.00	39.81
ATOM	88	CE	LYS	12	1.299	16.195	81.576	1.00	39.81
ATOM	89	NZ	LYS	12	-0.138	16.215	81.177	1.00	39.81
ATOM	90	C	LYS	12	6.140	12.168	81.415	1.00	39.27
ATOM	91	O	LYS	12	6.132	11.245	82.230	1.00	39.27
ATOM	92	N	MET	13	6.680	12.054	80.206	1.00	33.90
ATOM	93	CA	MET	13	7.278	10.794	79.780	1.00	33.90

ATOM	94	CB	MET	13	7.651	10.852	78.307	1.00	28.84
ATOM	95	CG	MET	13	6.478	10.988	77.344	1.00	28.84
ATOM	96	SD	MET	13	6.965	10.623	75.666	1.00	28.84
ATOM	97	CE	MET	13	5.331	10.780	74.827	1.00	28.84
ATOM	98	C	MET	13	8.508	10.455	80.620	1.00	33.90
ATOM	99	O	MET	13	8.751	9.289	80.918	1.00	33.90
ATOM	100	N	LYS	14	9.266	11.468	81.007	1.00	36.32
ATOM	101	CA	LYS	14	10.454	11.270	81.829	1.00	36.32
ATOM	102	CB	LYS	14	11.207	12.592	81.957	1.00	65.45
ATOM	103	CG	LYS	14	12.582	12.500	82.550	1.00	65.45
ATOM	104	CD	LYS	14	12.746	13.568	83.609	1.00	65.45
ATOM	105	CE	LYS	14	14.212	13.894	83.838	1.00	65.45
ATOM	106	NZ	LYS	14	14.426	14.645	85.109	1.00	65.45
ATOM	107	C	LYS	14	10.008	10.798	83.206	1.00	36.32
ATOM	108	O	LYS	14	10.636	9.943	83.824	1.00	36.32
ATOM	109	N	ARG	15	8.911	11.358	83.681	1.00	38.00
ATOM	110	CA	ARG	15	8.416	10.992	84.979	1.00	38.00
ATOM	111	CB	ARG	15	7.311	11.927	85.390	1.00	88.37
ATOM	112	CG	ARG	15	7.456	12.225	86.815	1.00	88.37
ATOM	113	CD	ARG	15	6.197	11.885	87.496	1.00	88.37
ATOM	114	NE	ARG	15	5.468	13.131	87.703	1.00	88.37
ATOM	115	CZ	ARG	15	4.230	13.240	88.152	1.00	88.37
ATOM	116	NH1	ARG	15	3.586	12.163	88.540	1.00	88.37
ATOM	117	NH2	ARG	15	3.650	14.435	88.213	1.00	88.37

ATOM	118	C	ARG	15	7.932	9.557	85.018	1.00	38.00
ATOM	119	O	ARG	15	8.193	8.825	85.982	1.00	38.00
ATOM	120	N	THR	16	7.236	9.156	83.963	1.00	33.54
ATOM	121	CA	THR	16	6.738	7.794	83.844	1.00	33.54
ATOM	122	CB	THR	16	6.013	7.603	82.499	1.00	26.11
ATOM	123	OG1	THR	16	4.716	8.216	82.562	1.00	26.11
ATOM	124	CG2	THR	16	5.899	6.117	82.148	1.00	26.11
ATOM	125	C	THR	16	7.931	6.841	83.902	1.00	33.54
ATOM	126	O	THR	16	7.956	5.911	84.704	1.00	33.54
ATOM	127	N	LEU	17	8.932	7.102	83.067	1.00	33.14
ATOM	128	CA	LEU	17	10.119	6.269	83.019	1.00	33.14
ATOM	129	CB	LEU	17	11.101	6.824	81.990	1.00	34.14
ATOM	130	CG	LEU	17	12.339	5.962	81.742	1.00	34.14
ATOM	131	CD1	LEU	17	11.951	4.644	81.086	1.00	34.14
ATOM	132	CD2	LEU	17	13.301	6.728	80.857	1.00	34.14
ATOM	133	C	LEU	17	10.783	6.171	84.390	1.00	33.14
ATOM	134	O	LEU	17	11.261	5.107	84.779	1.00	33.14
ATOM	135	N	GLU	18	10.802	7.268	85.135	1.00	32.45
ATOM	136	CA	GLU	18	11.419	7.237	86.454	1.00	32.45
ATOM	137	CB	GLU	18	11.607	8.661	86.990	1.00	59.96
ATOM	138	CG	GLU	18	12.984	9.224	86.630	1.00	59.96
ATOM	139	CD	GLU	18	13.076	10.747	86.698	1.00	59.96
ATOM	140	OE1	GLU	18	12.049	11.401	86.979	1.00	59.96
ATOM	141	OE2	GLU	18	14.185	11.287	86.460	1.00	59.96

ATOM	142	C	GLU	18	10.617	6.370	87.427	1.00 32.45
ATOM	143	O	GLU	18	11.190	5.687	88.282	1.00 32.45
ATOM	144	N	LYS	19	9.295	6.370	87.279	1.00 28.05
ATOM	145	CA	LYS	19	8.453	5.563	88.149	1.00 28.05
ATOM	146	CB	LYS	19	6.976	5.900	87.953	1.00 77.52
ATOM	147	CG	LYS	19	6.557	7.201	88.607	1.00 77.52
ATOM	148	CD	LYS	19	5.099	7.151	89.044	1.00 77.52
ATOM	149	CE	LYS	19	4.700	8.412	89.804	1.00 77.52
ATOM	150	NZ	LYS	19	3.354	8.277	90.440	1.00 77.52
ATOM	151	C	LYS	19	8.673	4.085	87.883	1.00 28.05
ATOM	152	O	LYS	19	8.729	3.275	88.813	1.00 28.05
ATOM	153	N	ILE	20	8.797	3.732	86.608	1.00 37.89
ATOM	154	CA	ILE	20	9.015	2.343	86.236	1.00 37.89
ATOM	155	CB	ILE	20	9.029	2.170	84.707	1.00 33.33
ATOM	156	CG2	ILE	20	9.407	0.744	84.348	1.00 33.33
ATOM	157	CG1	ILE	20	7.665	2.572	84.131	1.00 33.33
ATOM	158	CD1	ILE	20	6.488	1.863	84.752	1.00 33.33
ATOM	159	C	ILE	20	10.339	1.851	86.799	1.00 37.89
ATOM	160	O	ILE	20	10.406	0.793	87.421	1.00 37.89
ATOM	161	N	GLU	21	11.392	2.628	86.583	1.00 35.03
ATOM	162	CA	GLU	21	12.708	2.250	87.075	1.00 35.03
ATOM	163	CB	GLU	21	13.729	3.337	86.732	1.00 30.62
ATOM	164	CG	GLU	21	13.803	3.617	85.237	1.00 30.62
ATOM	165	CD	GLU	21	14.648	4.828	84.908	1.00 30.62

ATOM	166	OE1	GLU	21	14.706	5.755	85.736	1.00 30.62
ATOM	167	OE2	GLU	21	15.239	4.866	83.815	1.00 30.62
ATOM	168	C	GLU	21	12.628	2.055	88.578	1.00 35.03
ATOM	169	O	GLU	21	13.327	1.228	89.154	1.00 35.03
ATOM	170	N	ASP	22	11.746	2.821	89.206	1.00 38.35
ATOM	171	CA	ASP	22	11.560	2.756	90.643	1.00 38.35
ATOM	172	CB	ASP	22	10.706	3.946	91.088	1.00 38.81
ATOM	173	CG	ASP	22	10.825	4.230	92.567	1.00 38.81
ATOM	174	OD1	ASP	22	11.961	4.281	93.066	1.00 38.81
ATOM	175	OD2	ASP	22	9.784	4.416	93.230	1.00 38.81
ATOM	176	C	ASP	22	10.888	1.427	90.999	1.00 38.35
ATOM	177	O	ASP	22	11.355	0.698	91.875	1.00 38.35
ATOM	178	N	GLU	23	9.804	1.104	90.302	1.00 29.14
ATOM	179	CA	GLU	23	9.094	-0.142	90.546	1.00 29.14
ATOM	180	CB	GLU	23	7.875	-0.235	89.633	1.00 47.44
ATOM	181	CG	GLU	23	6.846	0.839	89.933	1.00 47.44
ATOM	182	CD	GLU	23	5.624	0.775	89.039	1.00 47.44
ATOM	183	OE1	GLU	23	4.619	1.425	89.368	1.00 47.44
ATOM	184	OE2	GLU	23	5.654	0.085	88.005	1.00 47.44
ATOM	185	C	GLU	23	10.008	-1.349	90.338	1.00 29.14
ATOM	186	O	GLU	23	10.007	-2.283	91.142	1.00 29.14
ATOM	187	N	LEU	24	10.796	-1.327	89.266	1.00 38.04
ATOM	188	CA	LEU	24	11.716	-2.423	88.981	1.00 38.04
ATOM	189	CB	LEU	24	12.360	-2.229	87.614	1.00 26.06

ATOM 190 CG LEU 24 11.366 -2.262 86.456 1.00 26.06
ATOM 191 CD1 LEU 24 12.097 -1.999 85.165 1.00 26.06
ATOM 192 CD2 LEU 24 10.657 -3.604 86.415 1.00 26.06
ATOM 193 C LEU 24 12.805 -2.520 90.039 1.00 38.04
ATOM 194 O LEU 24 13.360 -3.586 90.288 1.00 38.04
ATOM 195 N ARG 25 13.097 -1.393 90.666 1.00 38.24
ATOM 196 CA ARG 25 14.117 -1.324 91.690 1.00 38.24
ATOM 197 CB ARG 25 14.552 0.128 91.846 1.00 32.35
ATOM 198 CG ARG 25 15.401 0.400 93.059 1.00 32.35
ATOM 199 CD ARG 25 15.612 1.890 93.266 1.00 32.35
ATOM 200 NE ARG 25 15.485 2.205 94.680 1.00 32.35
ATOM 201 CZ ARG 25 14.386 2.692 95.246 1.00 32.35
ATOM 202 NH1 ARG 25 13.310 2.945 94.516 1.00 32.35
ATOM 203 NH2 ARG 25 14.352 2.872 96.557 1.00 32.35
ATOM 204 C ARG 25 13.648 -1.884 93.036 1.00 38.24
ATOM 205 O ARG 25 14.452 -2.427 93.795 1.00 38.24
ATOM 206 N LYS 26 12.352 -1.762 93.323 1.00 37.23
ATOM 207 CA LYS 26 11.792 -2.239 94.590 1.00 37.23
ATOM 208 CB LYS 26 10.685 -1.287 95.047 1.00 39.64
ATOM 209 CG LYS 26 11.150 0.165 95.116 1.00 39.64
ATOM 210 CD LYS 26 10.163 1.079 95.826 1.00 39.64
ATOM 211 CE LYS 26 8.877 1.234 95.054 1.00 39.64
ATOM 212 NZ LYS 26 8.005 2.257 95.699 1.00 39.64
ATOM 213 C LYS 26 11.258 -3.672 94.522 1.00 37.23

ATOM	214	O	LYS	26	10.856	-4.257	95.529	1.00 37.23
ATOM	215	N	MET	27	11.270	-4.218	93.318	1.00 33.15
ATOM	216	CA	MET	27	10.814	-5.566	93.008	1.00 33.15
ATOM	217	CB	MET	27	11.078	-5.799	91.535	1.00 37.03
ATOM	218	CG	MET	27	9.999	-6.456	90.761	1.00 37.03
ATOM	219	SD	MET	27	10.710	-6.822	89.162	1.00 37.03
ATOM	220	CE	MET	27	10.529	-8.604	89.133	1.00 37.03
ATOM	221	C	MET	27	11.586	-6.625	93.797	1.00 33.15
ATOM	222	O	MET	27	12.817	-6.535	93.910	1.00 33.15
ATOM	223	N	ARG	28	10.891	-7.632	94.332	1.00 34.95
ATOM	224	CA	ARG	28	11.600	-8.700	95.047	1.00 34.95
ATOM	225	CB	ARG	28	10.653	-9.617	95.831	1.00 37.05
ATOM	226	CG	ARG	28	11.379	-10.784	96.563	1.00 37.05
ATOM	227	CD	ARG	28	11.935	-11.825	95.587	1.00 37.05
ATOM	228	NE	ARG	28	12.853	-12.803	96.172	1.00 37.05
ATOM	229	CZ	ARG	28	12.485	-13.830	96.935	1.00 37.05
ATOM	230	NH1	ARG	28	11.205	-14.023	97.227	1.00 37.05
ATOM	231	NH2	ARG	28	13.397	-14.685	97.381	1.00 37.05
ATOM	232	C	ARG	28	12.284	-9.524	93.976	1.00 34.95
ATOM	233	O	ARG	28	11.663	-9.932	92.999	1.00 34.95
ATOM	234	N	THR	29	13.560	-9.792	94.176	1.00 38.90
ATOM	235	CA	THR	29	14.324	-10.557	93.211	1.00 38.90
ATOM	236	CB	THR	29	15.137	-9.563	92.332	1.00 35.61
ATOM	237	OG1	THR	29	14.492	-9.442	91.059	1.00 35.61

ATOM	238	CG2	THR	29	16.569	-9.974	92.161	1.00 35.61
ATOM	239	C	THR	29	15.184	-11.578	93.952	1.00 38.90
ATOM	240	O	THR	29	15.051	-11.739	95.164	1.00 38.90
ATOM	241	N	GLY	30	16.041	-12.288	93.233	1.00 39.05
ATOM	242	CA	GLY	30	16.877	-13.287	93.870	1.00 39.05
ATOM	243	C	GLY	30	17.824	-12.818	94.961	1.00 39.05
ATOM	244	O	GLY	30	18.097	-13.571	95.894	1.00 39.05
ATOM	245	N	LYS	31	18.327	-11.591	94.864	1.00 44.38
ATOM	246	CA	LYS	31	19.276	-11.095	95.859	1.00 44.38
ATOM	247	CB	LYS	31	20.463	-10.413	95.166	1.00 96.17
ATOM	248	CG	LYS	31	20.425	-8.883	95.169	1.00 96.17
ATOM	249	CD	LYS	31	19.350	-8.309	94.249	1.00 96.17
ATOM	250	CE	LYS	31	19.212	-6.796	94.447	1.00 96.17
ATOM	251	NZ	LYS	31	20.346	-6.211	95.237	1.00 96.17
ATOM	252	C	LYS	31	18.665	-10.138	96.876	1.00 44.38
ATOM	253	O	LYS	31	17.769	-9.360	96.553	1.00 44.38
ATOM	254	N	PRO	32	19.162	-10.176	98.121	1.00 38.22
ATOM	255	CD	PRO	32	20.333	-10.942	98.583	1.00 38.30
ATOM	256	CA	PRO	32	18.663	-9.308	99.190	1.00 38.22
ATOM	257	CB	PRO	32	19.512	-9.720	100.391	1.00 38.30
ATOM	258	CG	PRO	32	20.805	-10.115	99.765	1.00 38.30
ATOM	259	C	PRO	32	18.823	-7.833	98.853	1.00 38.22
ATOM	260	O	PRO	32	19.905	-7.386	98.482	1.00 38.22
ATOM	261	N	SER	33	17.734	-7.082	98.981	1.00 37.83

ATOM	262	CA	SER	33	17.753	-5.655	98.705	1.00 37.83
ATOM	263	CB	SER	33	17.385	-5.392	97.245	1.00 42.16
ATOM	264	OG	SER	33	17.475	-4.009	96.959	1.00 42.16
ATOM	265	C	SER	33	16.785	-4.914	99.626	1.00 37.83
ATOM	266	O	SER	33	15.588	-5.189	99.634	1.00 37.83
ATOM	267	N	PRO	34	17.302	-3.967	100.425	1.00 31.79
ATOM	268	CD	PRO	34	18.736	-3.767	100.705	1.00 21.38
ATOM	269	CA	PRO	34	16.450	-3.202	101.343	1.00 31.79
ATOM	270	CB	PRO	34	17.413	-2.226	102.022	1.00 21.38
ATOM	271	CG	PRO	34	18.762	-2.426	101.353	1.00 21.38
ATOM	272	C	PRO	34	15.304	-2.485	100.653	1.00 31.79
ATOM	273	O	PRO	34	14.306	-2.134	101.285	1.00 31.79
ATOM	274	N	ALA	35	15.435	-2.301	99.346	1.00 38.20
ATOM	275	CA	ALA	35	14.432	-1.607	98.561	1.00 38.20
ATOM	276	CB	ALA	35	14.956	-1.386	97.157	1.00 17.23
ATOM	277	C	ALA	35	13.072	-2.287	98.506	1.00 38.20
ATOM	278	O	ALA	35	12.067	-1.630	98.221	1.00 38.20
ATOM	279	N	ILE	36	13.024	-3.593	98.765	1.00 43.48
ATOM	280	CA	ILE	36	11.751	-4.315	98.723	1.00 43.48
ATOM	281	CB	ILE	36	11.950	-5.840	98.563	1.00 35.86
ATOM	282	CG2	ILE	36	12.611	-6.150	97.237	1.00 35.86
ATOM	283	CG1	ILE	36	12.815	-6.383	99.691	1.00 35.86
ATOM	284	CD1	ILE	36	13.127	-7.851	99.539	1.00 35.86
ATOM	285	C	ILE	36	10.902	-4.072	99.962	1.00 43.48

ATOM	286	O	ILE	36	9.721	-4.402	99.979	1.00 43.48
ATOM	287	N	LEU	37	11.502	-3.493	100.995	1.00 45.44
ATOM	288	CA	LEU	37	10.775	-3.214	102.222	1.00 45.44
ATOM	289	CB	LEU	37	11.697	-3.364	103.431	1.00 37.85
ATOM	290	CG	LEU	37	12.257	-4.779	103.613	1.00 37.85
ATOM	291	CD1	LEU	37	13.166	-4.833	104.832	1.00 37.85
ATOM	292	CD2	LEU	37	11.108	-5.760	103.761	1.00 37.85
ATOM	293	C	LEU	37	10.169	-1.822	102.205	1.00 45.44
ATOM	294	O	LEU	37	9.378	-1.474	103.075	1.00 45.44
ATOM	295	N	GLU	38	10.533	-1.029	101.206	1.00 35.29
ATOM	296	CA	GLU	38	10.024	0.327	101.086	1.00 35.29
ATOM	297	CB	GLU	38	10.727	1.047	99.938	1.00 35.02
ATOM	298	CG	GLU	38	12.126	1.507	100.291	1.00 35.02
ATOM	299	CD	GLU	38	12.859	2.111	99.118	1.00 35.02
ATOM	300	OE1	GLU	38	12.198	2.705	98.240	1.00 35.02
ATOM	301	OE2	GLU	38	14.103	2.005	99.084	1.00 35.02
ATOM	302	C	GLU	38	8.525	0.376	100.881	1.00 35.29
ATOM	303	O	GLU	38	7.898	1.409	101.088	1.00 35.29
ATOM	304	N	GLU	39	7.940	-0.744	100.485	1.00 43.94
ATOM	305	CA	GLU	39	6.503	-0.787	100.259	1.00 43.94
ATOM	306	CB	GLU	39	6.211	-1.291	98.846	1.00 81.69
ATOM	307	CG	GLU	39	6.456	-0.239	97.771	1.00 81.69
ATOM	308	CD	GLU	39	5.420	0.872	97.802	1.00 81.69
ATOM	309	OE1	GLU	39	4.242	0.586	97.505	1.00 81.69

ATOM	310	OE2	GLU	39	5.777	2.027	98.125	1.00	81.69
ATOM	311	C	GLU	39	5.783	-1.642	101.289	1.00	43.94
ATOM	312	O	GLU	39	4.582	-1.860	101.187	1.00	43.94
ATOM	313	N	ILE	40	6.519	-2.130	102.279	1.00	39.98
ATOM	314	CA	ILE	40	5.914	-2.929	103.332	1.00	39.98
ATOM	315	CB	ILE	40	6.874	-4.032	103.844	1.00	36.67
ATOM	316	CG2	ILE	40	6.193	-4.855	104.920	1.00	36.67
ATOM	317	CG1	ILE	40	7.284	-4.952	102.694	1.00	36.67
ATOM	318	CD1	ILE	40	6.116	-5.618	102.010	1.00	36.67
ATOM	319	C	ILE	40	5.591	-1.969	104.478	1.00	39.98
ATOM	320	O	ILE	40	6.490	-1.467	105.153	1.00	39.98
ATOM	321	N	LYS	41	4.305	-1.705	104.682	1.00	42.16
ATOM	322	CA	LYS	41	3.877	-0.797	105.732	1.00	42.16
ATOM	323	CB	LYS	41	2.794	0.146	105.205	1.00	51.75
ATOM	324	CG	LYS	41	3.189	0.926	103.966	1.00	51.75
ATOM	325	CD	LYS	41	1.962	1.566	103.350	1.00	51.75
ATOM	326	CE	LYS	41	2.274	2.250	102.032	1.00	51.75
ATOM	327	NZ	LYS	41	3.149	3.440	102.204	1.00	51.75
ATOM	328	C	LYS	41	3.346	-1.535	106.954	1.00	42.16
ATOM	329	O	LYS	41	2.775	-2.625	106.861	1.00	42.16
ATOM	330	N	VAL	42	3.527	-0.902	108.104	1.00	36.81
ATOM	331	CA	VAL	42	3.099	-1.440	109.377	1.00	36.81
ATOM	332	CB	VAL	42	4.351	-1.856	110.199	1.00	34.57
ATOM	333	CG1	VAL	42	4.204	-1.477	111.654	1.00	34.57

ATOM	334	CG2	VAL	42	4.578	-3.349	110.051	1.00	34.57
ATOM	335	C	VAL	42	2.313	-0.330	110.067	1.00	36.81
ATOM	336	O	VAL	42	2.684	0.836	109.975	1.00	36.81
ATOM	337	N	ASP	43	1.218	-0.676	110.734	1.00	42.28
ATOM	338	CA	ASP	43	0.416	0.329	111.424	1.00	42.28
ATOM	339	CB	ASP	43	-1.006	-0.189	111.671	1.00	62.32
ATOM	340	CG	ASP	43	-1.902	0.849	112.335	1.00	62.32
ATOM	341	OD1	ASP	43	-2.036	1.970	111.797	1.00	62.32
ATOM	342	OD2	ASP	43	-2.484	0.547	113.398	1.00	62.32
ATOM	343	C	ASP	43	1.099	0.650	112.743	1.00	42.28
ATOM	344	O	ASP	43	0.885	-0.027	113.744	1.00	42.28
ATOM	345	N	TYR	44	1.927	1.686	112.729	1.00	35.94
ATOM	346	CA	TYR	44	2.673	2.111	113.908	1.00	35.94
ATOM	347	CB	TYR	44	4.131	2.369	113.524	1.00	28.29
ATOM	348	CG	TYR	44	5.058	2.554	114.707	1.00	28.29
ATOM	349	CD1	TYR	44	5.766	3.746	114.883	1.00	28.29
ATOM	350	CE1	TYR	44	6.611	3.920	115.961	1.00	28.29
ATOM	351	CD2	TYR	44	5.230	1.533	115.650	1.00	28.29
ATOM	352	CE2	TYR	44	6.081	1.699	116.738	1.00	28.29
ATOM	353	CZ	TYR	44	6.769	2.893	116.881	1.00	28.29
ATOM	354	OH	TYR	44	7.623	3.064	117.938	1.00	28.29
ATOM	355	C	TYR	44	2.069	3.372	114.511	1.00	35.94
ATOM	356	O	TYR	44	2.100	4.439	113.898	1.00	35.94
ATOM	357	N	TYR	45	1.528	3.240	115.718	1.00	38.53

ATOM	358	CA	TYR	45	0.889	4.347	116.424	1.00	38.53
ATOM	359	CB	TYR	45	1.935	5.289	117.025	1.00	41.05
ATOM	360	CG	TYR	45	2.617	4.715	118.244	1.00	41.05
ATOM	361	CD1	TYR	45	3.728	3.879	118.120	1.00	41.05
ATOM	362	CE1	TYR	45	4.337	3.317	119.241	1.00	41.05
ATOM	363	CD2	TYR	45	2.144	4.998	119.532	1.00	41.05
ATOM	364	CE2	TYR	45	2.745	4.438	120.662	1.00	41.05
ATOM	365	CZ	TYR	45	3.851	3.610	120.506	1.00	41.05
ATOM	366	OH	TYR	45	4.461	3.056	121.610	1.00	41.05
ATOM	367	C	TYR	45	-0.086	5.148	115.573	1.00	38.53
ATOM	368	O	TYR	45	0.015	6.370	115.489	1.00	38.53
ATOM	369	N	GLY	46	-1.028	4.449	114.945	1.00	42.43
ATOM	370	CA	GLY	46	-2.034	5.107	114.128	1.00	42.43
ATOM	371	C	GLY	46	-1.673	5.501	112.705	1.00	42.43
ATOM	372	O	GLY	46	-2.542	5.949	111.957	1.00	42.43
ATOM	373	N	VAL	47	-0.416	5.327	112.313	1.00	41.54
ATOM	374	CA	VAL	47	0.010	5.700	110.969	1.00	41.54
ATOM	375	CB	VAL	47	0.992	6.898	111.037	1.00	35.99
ATOM	376	CG1	VAL	47	1.674	7.109	109.689	1.00	35.99
ATOM	377	CG2	VAL	47	0.237	8.148	111.451	1.00	35.99
ATOM	378	C	VAL	47	0.663	4.580	110.155	1.00	41.54
ATOM	379	O	VAL	47	1.623	3.958	110.604	1.00	41.54
ATOM	380	N	PRO	48	0.146	4.309	108.943	1.00	40.25
ATOM	381	CD	PRO	48	-1.000	4.969	108.294	1.00	34.00

ATOM	382	CA	PRO	48	0.709	3.259	108.080	1.00	40.25
ATOM	383	CB	PRO	48	-0.119	3.372	106.804	1.00	34.00
ATOM	384	CG	PRO	48	-1.437	3.928	107.293	1.00	34.00
ATOM	385	C	PRO	48	2.156	3.665	107.849	1.00	40.25
ATOM	386	O	PRO	48	2.420	4.700	107.237	1.00	40.25
ATOM	387	N	THR	49	3.096	2.855	108.316	1.00	32.23
ATOM	388	CA	THR	49	4.495	3.232	108.207	1.00	32.23
ATOM	389	CB	THR	49	5.047	3.475	109.622	1.00	26.24
ATOM	390	OG1	THR	49	4.090	4.237	110.368	1.00	26.24
ATOM	391	CG2	THR	49	6.375	4.212	109.574	1.00	26.24
ATOM	392	C	THR	49	5.434	2.271	107.485	1.00	32.23
ATOM	393	O	THR	49	5.561	1.108	107.867	1.00	32.23
ATOM	394	N	PRO	50	6.086	2.742	106.407	1.00	39.12
ATOM	395	CD	PRO	50	5.650	3.818	105.506	1.00	28.33
ATOM	396	CA	PRO	50	6.999	1.833	105.718	1.00	39.12
ATOM	397	CB	PRO	50	7.593	2.691	104.595	1.00	28.33
ATOM	398	CG	PRO	50	6.843	3.998	104.624	1.00	28.33
ATOM	399	C	PRO	50	8.045	1.349	106.709	1.00	39.12
ATOM	400	O	PRO	50	8.515	2.111	107.545	1.00	39.12
ATOM	401	N	VAL	51	8.404	0.076	106.603	1.00	34.69
ATOM	402	CA	VAL	51	9.356	-0.539	107.511	1.00	34.69
ATOM	403	CB	VAL	51	9.576	-2.006	107.122	1.00	36.79
ATOM	404	CG1	VAL	51	10.622	-2.623	108.005	1.00	36.79
ATOM	405	CG2	VAL	51	8.264	-2.773	107.254	1.00	36.79

ATOM	406	C	VAL	51	10.714	0.138	107.685	1.00	34.69
ATOM	407	O	VAL	51	11.158	0.336	108.817	1.00	34.69
ATOM	408	N	ASN	52	11.375	0.493	106.585	1.00	43.32
ATOM	409	CA	ASN	52	12.694	1.124	106.670	1.00	43.32
ATOM	410	CB	ASN	52	13.256	1.391	105.274	1.00	45.16
ATOM	411	CG	ASN	52	12.336	2.247	104.434	1.00	45.16
ATOM	412	OD1	ASN	52	11.261	1.808	104.033	1.00	45.16
ATOM	413	ND2	ASN	52	12.747	3.475	104.172	1.00	45.16
ATOM	414	C	ASN	52	12.698	2.423	107.458	1.00	43.32
ATOM	415	O	ASN	52	13.754	3.003	107.684	1.00	43.32
ATOM	416	N	GLN	53	11.522	2.883	107.868	1.00	41.50
ATOM	417	CA	GLN	53	11.415	4.120	108.635	1.00	41.50
ATOM	418	CB	GLN	53	10.109	4.841	108.305	1.00	32.22
ATOM	419	CG	GLN	53	10.163	5.659	107.035	1.00	32.22
ATOM	420	CD	GLN	53	8.818	6.244	106.664	1.00	32.22
ATOM	421	OE1	GLN	53	8.030	6.641	107.531	1.00	32.22
ATOM	422	NE2	GLN	53	8.551	6.320	105.363	1.00	32.22
ATOM	423	C	GLN	53	11.465	3.823	110.118	1.00	41.50
ATOM	424	O	GLN	53	11.719	4.713	110.937	1.00	41.50
ATOM	425	N	LEU	54	11.230	2.559	110.454	1.00	35.24
ATOM	426	CA	LEU	54	11.230	2.113	111.842	1.00	35.24
ATOM	427	CB	LEU	54	9.949	1.328	112.132	1.00	27.96
ATOM	428	CG	LEU	54	8.637	2.050	111.839	1.00	27.96
ATOM	429	CD1	LEU	54	7.462	1.130	112.101	1.00	27.96

ATOM	430	CD2	LEU	54	8.555	3.294	112.702	1.00	27.96
ATOM	431	C	LEU	54	12.427	1.223	112.144	1.00	35.24
ATOM	432	O	LEU	54	12.525	0.660	113.238	1.00	35.24
ATOM	433	N	ALA	55	13.341	1.091	111.188	1.00	26.03
ATOM	434	CA	ALA	55	14.481	0.218	111.411	1.00	26.03
ATOM	435	CB	ALA	55	14.038	-1.244	111.294	1.00	26.69
ATOM	436	C	ALA	55	15.638	0.457	110.479	1.00	26.03
ATOM	437	O	ALA	55	15.495	1.068	109.422	1.00	26.03
ATOM	438	N	THR	56	16.798	-0.025	110.897	1.00	37.20
ATOM	439	CA	THR	56	17.994	0.075	110.087	1.00	37.20
ATOM	440	CB	THR	56	19.261	0.167	110.946	1.00	41.17
ATOM	441	OG1	THR	56	19.399	-1.027	111.726	1.00	41.17
ATOM	442	CG2	THR	56	19.177	1.357	111.879	1.00	41.17
ATOM	443	C	THR	56	18.009	-1.238	109.317	1.00	37.20
ATOM	444	O	THR	56	17.785	-2.313	109.890	1.00	37.20
ATOM	445	N	ILE	57	18.244	-1.160	108.017	1.00	53.27
ATOM	446	CA	ILE	57	18.268	-2.364	107.214	1.00	53.27
ATOM	447	CB	ILE	57	17.193	-2.321	106.131	1.00	37.44
ATOM	448	CG2	ILE	57	17.215	-3.612	105.330	1.00	37.44
ATOM	449	CG1	ILE	57	15.828	-2.101	106.789	1.00	37.44
ATOM	450	CD1	ILE	57	14.665	-2.037	105.810	1.00	37.44
ATOM	451	C	ILE	57	19.632	-2.521	106.579	1.00	53.27
ATOM	452	O	ILE	57	20.124	-1.626	105.899	1.00	53.27
ATOM	453	N	SER	58	20.248	-3.668	106.820	1.00	58.33

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ATOM    454  CA  SER    58      21.571  -3.954 106.287  1.00 58.33
ATOM    455  CB  SER    58      22.609  -3.776 107.395  1.00 54.61
ATOM    456  OG  SER    58      22.130  -4.320 108.610  1.00 54.61
ATOM    457  C   SER    58      21.619  -5.371 105.735  1.00 58.33
ATOM    458  O   SER    58      20.691  -6.142 105.952  1.00 58.33
ATOM    459  N   ILE    59      22.696  -5.719 105.047  1.00 52.33
ATOM    460  CA  ILE    59      22.809  -7.036 104.463  1.00 52.33
ATOM    461  CB  ILE    59      23.345  -6.880 103.002  1.00 41.35
ATOM    462  CG2 ILE    59      23.395  -8.205 102.308  1.00 41.35
ATOM    463  CG1 ILE    59      22.416  -5.923 102.242  1.00 41.35
ATOM    464  CD1 ILE    59      22.248  -6.171 100.721  1.00 41.35
ATOM    465  C   ILE    59      23.718  -7.878 105.353  1.00 52.33
ATOM    466  O   ILE    59      24.936  -7.696 105.361  1.00 52.33
ATOM    467  N   SER    60      23.109  -8.762 106.143  1.00 60.00
ATOM    468  CA  SER    60      23.886  -9.591 107.064  1.00 60.00
ATOM    469  CB  SER    60      23.010 -10.539 107.912  1.00 60.63
ATOM    470  OG  SER    60      22.600 -11.739 107.279  1.00 60.63
ATOM    471  C   SER    60      24.778 -10.428 106.252  1.00 60.00
ATOM    472  O   SER    60      25.950 -10.189 106.125  1.00 60.00
ATOM    473  N   GLU    61      24.235 -11.488 105.732  1.00 87.30
ATOM    474  CA  GLU    61      25.102 -12.210 104.852  1.00 87.30
ATOM    475  CB  GLU    61      25.179 -13.704 105.187  1.00 88.30
ATOM    476  CG  GLU    61      23.932 -14.400 105.050  1.00 88.30
ATOM    477  CD  GLU    61      24.112 -15.569 104.196  1.00 88.30
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ATOM 478 OE1 GLU 61 23.382 -15.647 103.202 1.00 88.30
ATOM 479 OE2 GLU 61 24.982 -16.404 104.514 1.00 88.30
ATOM 480 C GLU 61 24.601 -11.951 103.471 1.00 87.30
ATOM 481 O GLU 61 23.600 -11.303 103.276 1.00 87.30
ATOM 482 N GLU 62 25.450 -12.404 102.552 1.00 61.89
ATOM 483 CA GLU 62 25.257 -12.362 101.100 1.00 61.89
ATOM 484 CB GLU 62 26.426 -13.116 100.473 1.00 60.63
ATOM 485 CG GLU 62 27.704 -12.340 100.884 1.00 60.63
ATOM 486 CD GLU 62 28.278 -12.647 102.306 1.00 60.63
ATOM 487 OE1 GLU 62 27.634 -13.284 103.188 1.00 60.63
ATOM 488 OE2 GLU 62 29.433 -12.215 102.521 1.00 60.63
ATOM 489 C GLU 62 23.847 -12.744 100.622 1.00 61.89
ATOM 490 O GLU 62 23.455 -12.418 99.550 1.00 61.89
ATOM 491 N ARG 63 23.074 -13.357 101.491 1.00 43.14
ATOM 492 CA ARG 63 21.691 -13.788 101.154 1.00 43.14
ATOM 493 CB ARG 63 21.592 -15.314 101.038 1.00 76.81
ATOM 494 CG ARG 63 22.506 -15.940 100.016 1.00 76.81
ATOM 495 CD ARG 63 21.645 -16.662 99.020 1.00 76.81
ATOM 496 NE ARG 63 22.409 -17.245 97.930 1.00 76.81
ATOM 497 CZ ARG 63 21.892 -17.540 96.743 1.00 76.81
ATOM 498 NH1 ARG 63 20.610 -17.305 96.498 1.00 76.81
ATOM 499 NH2 ARG 63 22.663 -18.049 95.793 1.00 76.81
ATOM 500 C ARG 63 20.705 -13.359 102.198 1.00 43.14
ATOM 501 O ARG 63 19.544 -13.764 102.165 1.00 43.14

ATOM 502 N THR 64 21.161 -12.500 103.109 1.00 37.34
ATOM 503 CA THR 64 20.310 -12.052 104.183 1.00 37.34
ATOM 504 CB THR 64 20.799 -12.615 105.553 1.00 39.55
ATOM 505 OG1 THR 64 20.904 -14.040 105.505 1.00 39.55
ATOM 506 CG2 THR 64 19.819 -12.237 106.662 1.00 39.55
ATOM 507 C THR 64 20.118 -10.553 104.378 1.00 37.34
ATOM 508 O THR 64 21.053 -9.760 104.271 1.00 37.34
ATOM 509 N LEU 65 18.880 -10.188 104.695 1.00 39.85
ATOM 510 CA LEU 65 18.516 -8.812 104.998 1.00 39.85
ATOM 511 CB LEU 65 17.236 -8.403 104.271 1.00 33.79
ATOM 512 CG LEU 65 17.295 -7.751 102.896 1.00 33.79
ATOM 513 CD1 LEU 65 15.874 -7.601 102.368 1.00 33.79
ATOM 514 CD2 LEU 65 17.989 -6.408 102.997 1.00 33.79
ATOM 515 C LEU 65 18.225 -8.806 106.489 1.00 39.85
ATOM 516 O LEU 65 17.402 -9.585 106.956 1.00 39.85
ATOM 517 N VAL 66 18.902 -7.950 107.238 1.00 42.65
ATOM 518 CA VAL 66 18.649 -7.864 108.666 1.00 42.65
ATOM 519 CB VAL 66 19.958 -7.878 109.478 1.00 46.94
ATOM 520 CG1 VAL 66 19.654 -7.792 110.951 1.00 46.94
ATOM 521 CG2 VAL 66 20.718 -9.152 109.203 1.00 46.94
ATOM 522 C VAL 66 17.904 -6.558 108.909 1.00 42.65
ATOM 523 O VAL 66 18.366 -5.487 108.514 1.00 42.65
ATOM 524 N ILE 67 16.740 -6.669 109.543 1.00 31.17
ATOM 525 CA ILE 67 15.890 -5.526 109.844 1.00 31.17

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ATOM    526  CB  ILE    67      14.441  -5.790 109.421  1.00 22.89
ATOM    527  CG2 ILE    67      13.618  -4.516 109.546  1.00 22.89
ATOM    528  CG1 ILE    67      14.401  -6.336 107.987  1.00 22.89
ATOM    529  CD1 ILE    67      13.024  -6.819 107.571  1.00 22.89
ATOM    530  C   ILE    67      15.888  -5.308 111.345  1.00 31.17
ATOM    531  O   ILE    67      15.204  -6.032 112.073  1.00 31.17
ATOM    532  N   LYS    68      16.639  -4.309 111.806  1.00 42.20
ATOM    533  CA  LYS    68      16.719  -4.005 113.229  1.00 42.20
ATOM    534  CB  LYS    68      18.180  -3.819 113.623  1.00 56.36
ATOM    535  CG  LYS    68      18.405  -3.640 115.105  1.00 56.36
ATOM    536  CD  LYS    68      19.850  -3.923 115.458  1.00 56.36
ATOM    537  CE  LYS    68      20.079  -3.792 116.952  1.00 56.36
ATOM    538  NZ  LYS    68      19.129  -4.626 117.749  1.00 56.36
ATOM    539  C   LYS    68      15.913  -2.768 113.634  1.00 42.20
ATOM    540  O   LYS    68      16.306  -1.636 113.360  1.00 42.20
ATOM    541  N   PRO    69      14.763  -2.970 114.283  1.00 39.13
ATOM    542  CD  PRO    69      14.029  -4.240 114.428  1.00 36.24
ATOM    543  CA  PRO    69      13.936  -1.837 114.709  1.00 39.13
ATOM    544  CB  PRO    69      12.530  -2.410 114.649  1.00 36.24
ATOM    545  CG  PRO    69      12.750  -3.805 115.143  1.00 36.24
ATOM    546  C   PRO    69      14.320  -1.409 116.123  1.00 39.13
ATOM    547  O   PRO    69      14.665  -2.247 116.950  1.00 39.13
ATOM    548  N   TRP    70      14.274  -0.112 116.406  1.00 35.31
ATOM    549  CA  TRP    70      14.625   0.359 117.742  1.00 35.31
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ATOM	550	CB	TRP	70	14.990	1.843	117.719	1.00	54.18
ATOM	551	CG	TRP	70	14.618	2.524	116.449	1.00	54.18
ATOM	552	CD2	TRP	70	13.375	3.187	116.177	1.00	54.18
ATOM	553	CE2	TRP	70	13.461	3.663	114.844	1.00	54.18
ATOM	554	CE3	TRP	70	12.220	3.423	116.922	1.00	54.18
ATOM	555	CD1	TRP	70	15.371	2.620	115.312	1.00	54.18
ATOM	556	NE1	TRP	70	14.671	3.305	114.345	1.00	54.18
ATOM	557	CZ2	TRP	70	12.400	4.366	114.251	1.00	54.18
ATOM	558	CZ3	TRP	70	11.174	4.121	116.329	1.00	54.18
ATOM	559	CH2	TRP	70	11.276	4.587	115.003	1.00	54.18
ATOM	560	C	TRP	70	13.477	0.111	118.713	1.00	35.31
ATOM	561	O	TRP	70	13.690	0.011	119.913	1.00	35.31
ATOM	562	N	ASP	71	12.259	0.018	118.194	1.00	38.62
ATOM	563	CA	ASP	71	11.097	-0.252	119.036	1.00	38.62
ATOM	564	CB	ASP	71	9.897	0.580	118.587	1.00	33.95
ATOM	565	CG	ASP	71	8.667	0.323	119.436	1.00	33.95
ATOM	566	OD1	ASP	71	8.604	-0.741	120.086	1.00	33.95
ATOM	567	OD2	ASP	71	7.754	1.174	119.445	1.00	33.95
ATOM	568	C	ASP	71	10.785	-1.740	118.869	1.00	38.62
ATOM	569	O	ASP	71	10.037	-2.142	117.972	1.00	38.62
ATOM	570	N	LYS	72	11.361	-2.553	119.741	1.00	42.47
ATOM	571	CA	LYS	72	11.190	-3.990	119.661	1.00	42.47
ATOM	572	CB	LYS	72	11.903	-4.650	120.843	1.00	41.13
ATOM	573	CG	LYS	72	13.420	-4.457	120.777	1.00	41.13

ATOM 574 CD LYS 72 13.964 -5.079 119.500 1.00 41.13
ATOM 575 CE LYS 72 15.041 -4.235 118.810 1.00 41.13
ATOM 576 NZ LYS 72 16.328 -4.168 119.527 1.00 41.13
ATOM 577 C LYS 72 9.774 -4.530 119.516 1.00 42.47
ATOM 578 O LYS 72 9.600 -5.690 119.148 1.00 42.47
ATOM 579 N SER 73 8.759 -3.719 119.781 1.00 36.48
ATOM 580 CA SER 73 7.390 -4.212 119.631 1.00 36.48
ATOM 581 CB SER 73 6.401 -3.336 120.403 1.00 49.51
ATOM 582 OG SER 73 6.288 -2.045 119.832 1.00 49.51
ATOM 583 C SER 73 7.030 -4.206 118.153 1.00 36.48
ATOM 584 O SER 73 6.006 -4.745 117.750 1.00 36.48
ATOM 585 N VAL 74 7.891 -3.593 117.352 1.00 41.78
ATOM 586 CA VAL 74 7.690 -3.487 115.910 1.00 41.78
ATOM 587 CB VAL 74 8.562 -2.340 115.332 1.00 35.01
ATOM 588 CG1 VAL 74 8.640 -2.425 113.811 1.00 35.01
ATOM 589 CG2 VAL 74 7.982 -1.009 115.754 1.00 35.01
ATOM 590 C VAL 74 7.997 -4.791 115.176 1.00 41.78
ATOM 591 O VAL 74 7.475 -5.032 114.085 1.00 41.78
ATOM 592 N LEU 75 8.842 -5.626 115.778 1.00 35.55
ATOM 593 CA LEU 75 9.212 -6.914 115.191 1.00 35.55
ATOM 594 CB LEU 75 10.063 -7.707 116.180 1.00 30.58
ATOM 595 CG LEU 75 11.420 -7.091 116.509 1.00 30.58
ATOM 596 CD1 LEU 75 12.024 -7.786 117.707 1.00 30.58
ATOM 597 CD2 LEU 75 12.338 -7.213 115.288 1.00 30.58

ATOM 598 C LEU 75 7.968 -7.725 114.828 1.00 35.55
ATOM 599 O LEU 75 7.830 -8.220 113.714 1.00 35.55
ATOM 600 N SER 76 7.057 -7.850 115.777 1.00 35.44
ATOM 601 CA SER 76 5.843 -8.601 115.540 1.00 35.44
ATOM 602 CB SER 76 4.954 -8.545 116.782 1.00 41.93
ATOM 603 OG SER 76 3.829 -9.382 116.623 1.00 41.93
ATOM 604 C SER 76 5.087 -8.062 114.321 1.00 35.44
ATOM 605 O SER 76 4.649 -8.831 113.462 1.00 35.44
ATOM 606 N LEU 77 4.951 -6.738 114.251 1.00 39.58
ATOM 607 CA LEU 77 4.246 -6.078 113.152 1.00 39.58
ATOM 608 CB LEU 77 4.097 -4.577 113.446 1.00 32.60
ATOM 609 CG LEU 77 3.248 -4.216 114.681 1.00 32.60
ATOM 610 CD1 LEU 77 3.301 -2.715 114.936 1.00 32.60
ATOM 611 CD2 LEU 77 1.808 -4.676 114.476 1.00 32.60
ATOM 612 C LEU 77 4.941 -6.277 111.805 1.00 39.58
ATOM 613 O LEU 77 4.288 -6.510 110.787 1.00 39.58
ATOM 614 N ILE 78 6.263 -6.181 111.792 1.00 38.16
ATOM 615 CA ILE 78 6.996 -6.362 110.553 1.00 38.16
ATOM 616 CB ILE 78 8.501 -6.146 110.753 1.00 31.39
ATOM 617 CG2 ILE 78 9.240 -6.525 109.478 1.00 31.39
ATOM 618 CG1 ILE 78 8.773 -4.691 111.153 1.00 31.39
ATOM 619 CD1 ILE 78 10.212 -4.413 111.518 1.00 31.39
ATOM 620 C ILE 78 6.779 -7.774 110.031 1.00 38.16
ATOM 621 O ILE 78 6.590 -7.985 108.833 1.00 38.16

ATOM	622	N	GLU	79	6.802	-8.737	110.942	1.00	34.51
ATOM	623	CA	GLU	79	6.618	-10.136	110.599	1.00	34.51
ATOM	624	CB	GLU	79	6.802	-10.982	111.857	1.00	43.51
ATOM	625	CG	GLU	79	6.587	-12.471	111.665	1.00	43.51
ATOM	626	CD	GLU	79	6.869	-13.261	112.931	1.00	43.51
ATOM	627	OE1	GLU	79	6.782	-14.505	112.889	1.00	43.51
ATOM	628	OE2	GLU	79	7.178	-12.642	113.972	1.00	43.51
ATOM	629	C	GLU	79	5.243	-10.387	109.987	1.00	34.51
ATOM	630	O	GLU	79	5.115	-11.058	108.961	1.00	34.51
ATOM	631	N	LYS	80	4.215	-9.846	110.625	1.00	41.03
ATOM	632	CA	LYS	80	2.845	-9.994	110.153	1.00	41.03
ATOM	633	CB	LYS	80	1.905	-9.247	111.090	1.00	63.82
ATOM	634	CG	LYS	80	0.758	-10.055	111.630	1.00	63.82
ATOM	635	CD	LYS	80	1.056	-10.548	113.035	1.00	63.82
ATOM	636	CE	LYS	80	-0.230	-10.873	113.785	1.00	63.82
ATOM	637	NZ	LYS	80	0.064	-11.206	115.204	1.00	63.82
ATOM	638	C	LYS	80	2.695	-9.401	108.753	1.00	41.03
ATOM	639	O	LYS	80	2.128	-10.022	107.847	1.00	41.03
ATOM	640	N	ALA	81	3.198	-8.177	108.601	1.00	39.91
ATOM	641	CA	ALA	81	3.131	-7.437	107.351	1.00	39.91
ATOM	642	CB	ALA	81	3.738	-6.065	107.535	1.00	28.92
ATOM	643	C	ALA	81	3.831	-8.161	106.216	1.00	39.91
ATOM	644	O	ALA	81	3.358	-8.145	105.077	1.00	39.91
ATOM	645	N	ILE	82	4.966	-8.782	106.516	1.00	31.35

ATOM	646	CA	ILE	82	5.691	-9.509	105.490	1.00	31.35
ATOM	647	CB	ILE	82	7.103	-9.899	105.959	1.00	31.86
ATOM	648	CG2	ILE	82	7.746	-10.830	104.943	1.00	31.86
ATOM	649	CG1	ILE	82	7.948	-8.634	106.151	1.00	31.86
ATOM	650	CD1	ILE	82	9.372	-8.905	106.571	1.00	31.86
ATOM	651	C	ILE	82	4.919	-10.759	105.095	1.00	31.35
ATOM	652	O	ILE	82	4.763	-11.041	103.904	1.00	31.35
ATOM	653	N	ASN	83	4.420	-11.503	106.080	1.00	44.39
ATOM	654	CA	ASN	83	3.660	-12.712	105.778	1.00	44.39
ATOM	655	CB	ASN	83	3.253	-13.449	107.064	1.00	33.35
ATOM	656	CG	ASN	83	4.427	-14.137	107.747	1.00	33.35
ATOM	657	OD1	ASN	83	5.289	-14.727	107.092	1.00	33.35
ATOM	658	ND2	ASN	83	4.454	-14.078	109.075	1.00	33.35
ATOM	659	C	ASN	83	2.411	-12.393	104.949	1.00	44.39
ATOM	660	O	ASN	83	1.936	-13.236	104.186	1.00	44.39
ATOM	661	N	ALA	84	1.888	-11.178	105.086	1.00	41.69
ATOM	662	CA	ALA	84	0.698	-10.777	104.334	1.00	41.69
ATOM	663	CB	ALA	84	-0.061	-9.684	105.090	1.00	35.44
ATOM	664	C	ALA	84	1.026	-10.294	102.919	1.00	41.69
ATOM	665	O	ALA	84	0.147	-10.231	102.059	1.00	41.69
ATOM	666	N	SER	85	2.285	-9.945	102.673	1.00	47.02
ATOM	667	CA	SER	85	2.681	-9.478	101.346	1.00	47.02
ATOM	668	CB	SER	85	3.952	-8.645	101.439	1.00	37.52
ATOM	669	OG	SER	85	5.037	-9.457	101.836	1.00	37.52

ATOM 670 C SER 85 2.931 -10.663 100.421 1.00 47.02
ATOM 671 O SER 85 2.773 -11.815 100.820 1.00 47.02
ATOM 672 N ASP 86 3.330 -10.380 99.188 1.00 55.10
ATOM 673 CA ASP 86 3.600 -11.438 98.226 1.00 55.10
ATOM 674 CB ASP 86 2.856 -11.159 96.913 1.00 66.61
ATOM 675 CG ASP 86 3.232 -9.821 96.292 1.00 66.61
ATOM 676 OD1 ASP 86 4.108 -9.119 96.841 1.00 66.61
ATOM 677 OD2 ASP 86 2.647 -9.468 95.244 1.00 66.61
ATOM 678 C ASP 86 5.093 -11.593 97.955 1.00 55.10
ATOM 679 O ASP 86 5.492 -12.136 96.921 1.00 55.10
ATOM 680 N LEU 87 5.916 -11.127 98.889 1.00 40.02
ATOM 681 CA LEU 87 7.368 -11.201 98.741 1.00 40.02
ATOM 682 CB LEU 87 8.056 -10.528 99.931 1.00 53.14
ATOM 683 CG LEU 87 7.867 -9.019 100.053 1.00 53.14
ATOM 684 CD1 LEU 87 8.549 -8.512 101.314 1.00 53.14
ATOM 685 CD2 LEU 87 8.446 -8.348 98.815 1.00 53.14
ATOM 686 C LEU 87 7.924 -12.612 98.596 1.00 40.02
ATOM 687 O LEU 87 8.967 -12.812 97.967 1.00 40.02
ATOM 688 N GLY 88 7.233 -13.588 99.176 1.00 39.77
ATOM 689 CA GLY 88 7.722 -14.951 99.109 1.00 39.77
ATOM 690 C GLY 88 8.924 -15.046 100.028 1.00 39.77
ATOM 691 O GLY 88 9.905 -15.733 99.740 1.00 39.77
ATOM 692 N LEU 89 8.848 -14.319 101.138 1.00 36.60
ATOM 693 CA LEU 89 9.907 -14.303 102.124 1.00 36.60

ATOM 694 CB LEU 89 10.522 -12.909 102.221 1.00 41.00
ATOM 695 CG LEU 89 11.343 -12.412 101.033 1.00 41.00
ATOM 696 CD1 LEU 89 11.804 -10.996 101.312 1.00 41.00
ATOM 697 CD2 LEU 89 12.539 -13.321 100.797 1.00 41.00
ATOM 698 C LEU 89 9.348 -14.720 103.477 1.00 36.60
ATOM 699 O LEU 89 8.186 -14.464 103.792 1.00 36.60
ATOM 700 N ASN 90 10.185 -15.368 104.275 1.00 32.75
ATOM 701 CA ASN 90 9.774 -15.834 105.585 1.00 32.75
ATOM 702 CB ASN 90 9.973 -17.343 105.682 1.00 37.39
ATOM 703 CG ASN 90 9.128 -18.104 104.682 1.00 37.39
ATOM 704 OD1 ASN 90 7.902 -18.176 104.813 1.00 37.39
ATOM 705 ND2 ASN 90 9.776 -18.671 103.670 1.00 37.39
ATOM 706 C ASN 90 10.593 -15.141 106.651 1.00 32.75
ATOM 707 O ASN 90 11.804 -15.323 106.731 1.00 32.75
ATOM 708 N PRO 91 9.941 -14.330 107.484 1.00 35.24
ATOM 709 CD PRO 91 8.503 -14.012 107.514 1.00 26.32
ATOM 710 CA PRO 91 10.657 -13.620 108.543 1.00 35.24
ATOM 711 CB PRO 91 9.597 -12.670 109.088 1.00 26.32
ATOM 712 CG PRO 91 8.339 -13.443 108.899 1.00 26.32
ATOM 713 C PRO 91 11.212 -14.545 109.621 1.00 35.24
ATOM 714 O PRO 91 10.577 -15.528 110.004 1.00 35.24
ATOM 715 N ILE 92 12.406 -14.224 110.099 1.00 39.44
ATOM 716 CA ILE 92 13.050 -14.988 111.156 1.00 39.44
ATOM 717 CB ILE 92 14.442 -15.457 110.736 1.00 30.73

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ATOM    718  CG2 ILE    92      15.044 -16.318 111.833  1.00 30.73
ATOM    719  CG1 ILE    92      14.357 -16.256 109.440  1.00 30.73
ATOM    720  CD1 ILE    92      15.720 -16.628 108.899  1.00 30.73
ATOM    721  C   ILE    92      13.199 -14.016 112.323  1.00 39.44
ATOM    722  O   ILE    92      14.117 -13.200 112.347  1.00 39.44
ATOM    723  N   ASN    93      12.291 -14.109 113.287  1.00 37.86
ATOM    724  CA  ASN    93      12.275 -13.220 114.441  1.00 37.86
ATOM    725  CB  ASN    93      10.818 -12.947 114.804  1.00 26.31
ATOM    726  CG  ASN    93      10.660 -11.871 115.856  1.00 26.31
ATOM    727  OD1 ASN    93      11.627 -11.440 116.482  1.00 26.31
ATOM    728  ND2 ASN    93       9.426 -11.431 116.059  1.00 26.31
ATOM    729  C   ASN    93      13.015 -13.760 115.668  1.00 37.86
ATOM    730  O   ASN    93      12.538 -14.690 116.321  1.00 37.86
ATOM    731  N   ASP    94      14.166 -13.174 115.995  1.00 43.86
ATOM    732  CA  ASP    94      14.926 -13.613 117.167  1.00 43.86
ATOM    733  CB  ASP    94      16.429 -13.647 116.866  1.00 48.91
ATOM    734  CG  ASP    94      17.003 -12.274 116.625  1.00 48.91
ATOM    735  OD1 ASP    94      18.236 -12.162 116.455  1.00 48.91
ATOM    736  OD2 ASP    94      16.220 -11.305 116.603  1.00 48.91
ATOM    737  C   ASP    94      14.661 -12.696 118.366  1.00 43.86
ATOM    738  O   ASP    94      15.416 -12.690 119.339  1.00 43.86
ATOM    739  N   GLY    95      13.589 -11.914 118.286  1.00 38.45
ATOM    740  CA  GLY    95      13.233 -11.031 119.381  1.00 38.45
ATOM    741  C   GLY    95      14.025  -9.743 119.451  1.00 38.45
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ATOM	742	O	GLY	95	13.756	-8.886	120.293	1.00 38.45
ATOM	743	N	ASN	96	15.008	-9.606	118.569	1.00 49.36
ATOM	744	CA	ASN	96	15.841	-8.410	118.525	1.00 49.36
ATOM	745	CB	ASN	96	17.296	-8.751	118.860	1.00 40.40
ATOM	746	CG	ASN	96	18.208	-7.532	118.808	1.00 40.40
ATOM	747	OD1	ASN	96	19.369	-7.620	118.399	1.00 40.40
ATOM	748	ND2	ASN	96	17.686	-6.388	119.232	1.00 40.40
ATOM	749	C	ASN	96	15.789	-7.849	117.116	1.00 49.36
ATOM	750	O	ASN	96	15.489	-6.679	116.901	1.00 49.36
ATOM	751	N	VAL	97	16.079	-8.716	116.158	1.00 44.00
ATOM	752	CA	VAL	97	16.105	-8.340	114.759	1.00 44.00
ATOM	753	CB	VAL	97	17.561	-8.390	114.253	1.00 36.85
ATOM	754	CG1	VAL	97	17.828	-9.690	113.506	1.00 36.85
ATOM	755	CG2	VAL	97	17.843	-7.197	113.413	1.00 36.85
ATOM	756	C	VAL	97	15.223	-9.276	113.923	1.00 44.00
ATOM	757	O	VAL	97	14.713	-10.279	114.422	1.00 44.00
ATOM	758	N	ILE	98	15.033	-8.938	112.653	1.00 37.43
ATOM	759	CA	ILE	98	14.240	-9.772	111.758	1.00 37.43
ATOM	760	CB	ILE	98	12.936	-9.084	111.332	1.00 38.61
ATOM	761	CG2	ILE	98	12.439	-9.659	110.012	1.00 38.61
ATOM	762	CG1	ILE	98	11.885	-9.281	112.420	1.00 38.61
ATOM	763	CD1	ILE	98	10.495	-8.910	111.987	1.00 38.61
ATOM	764	C	ILE	98	15.036	-10.105	110.515	1.00 37.43
ATOM	765	O	ILE	98	15.270	-9.236	109.677	1.00 37.43

ATOM	766	N	ARG	99	15.458	-11.365	110.410	1.00 30.52
ATOM	767	CA	ARG	99	16.232	-11.822	109.263	1.00 30.52
ATOM	768	CB	ARG	99	17.137	-13.004	109.625	1.00 50.62
ATOM	769	CG	ARG	99	18.429	-12.643	110.328	1.00 50.62
ATOM	770	CD	ARG	99	19.445	-13.758	110.162	1.00 50.62
ATOM	771	NE	ARG	99	18.996	-15.003	110.777	1.00 50.62
ATOM	772	CZ	ARG	99	19.434	-16.213	110.440	1.00 50.62
ATOM	773	NH1	ARG	99	20.340	-16.363	109.479	1.00 50.62
ATOM	774	NH2	ARG	99	18.962	-17.277	111.074	1.00 50.62
ATOM	775	C	ARG	99	15.307	-12.244	108.151	1.00 30.52
ATOM	776	O	ARG	99	14.242	-12.809	108.385	1.00 30.52
ATOM	777	N	LEU	100	15.729	-11.957	106.932	1.00 33.80
ATOM	778	CA	LEU	100	14.973	-12.307	105.743	1.00 33.80
ATOM	779	CB	LEU	100	14.399	-11.042	105.117	1.00 34.63
ATOM	780	CG	LEU	100	12.890	-10.941	104.938	1.00 34.63
ATOM	781	CD1	LEU	100	12.167	-11.437	106.178	1.00 34.63
ATOM	782	CD2	LEU	100	12.542	-9.493	104.636	1.00 34.63
ATOM	783	C	LEU	100	16.011	-12.928	104.829	1.00 33.80
ATOM	784	O	LEU	100	16.829	-12.225	104.247	1.00 33.80
ATOM	785	N	VAL	101	16.006	-14.248	104.725	1.00 40.27
ATOM	786	CA	VAL	101	16.989	-14.911	103.887	1.00 40.27
ATOM	787	CB	VAL	101	17.377	-16.271	104.470	1.00 42.80
ATOM	788	CG1	VAL	101	18.462	-16.902	103.621	1.00 42.80
ATOM	789	CG2	VAL	101	17.867	-16.097	105.888	1.00 42.80

ATOM 790 C VAL 101 16.481 -15.087 102.467 1.00 40.27
ATOM 791 O VAL 101 15.288 -15.295 102.248 1.00 40.27
ATOM 792 N PHE 102 17.396 -14.988 101.506 1.00 42.62
ATOM 793 CA PHE 102 17.065 -15.120 100.091 1.00 42.62
ATOM 794 CB PHE 102 17.504 -13.870 99.325 1.00 35.61
ATOM 795 CG PHE 102 16.807 -12.614 99.755 1.00 35.61
ATOM 796 CD1 PHE 102 17.012 -12.089 101.026 1.00 35.61
ATOM 797 CD2 PHE 102 15.936 -11.955 98.889 1.00 35.61
ATOM 798 CE1 PHE 102 16.365 -10.926 101.427 1.00 35.61
ATOM 799 CE2 PHE 102 15.286 -10.793 99.284 1.00 35.61
ATOM 800 CZ PHE 102 15.498 -10.280 100.553 1.00 35.61
ATOM 801 C PHE 102 17.758 -16.330 99.481 1.00 42.62
ATOM 802 O PHE 102 18.853 -16.210 98.938 1.00 42.62
ATOM 803 N PRO 103 17.123 -17.509 99.542 1.00 35.91
ATOM 804 CD PRO 103 15.787 -17.769 100.101 1.00 36.33
ATOM 805 CA PRO 103 17.710 -18.739 98.983 1.00 35.91
ATOM 806 CB PRO 103 16.665 -19.811 99.313 1.00 36.33
ATOM 807 CG PRO 103 15.900 -19.221 100.488 1.00 36.33
ATOM 808 C PRO 103 17.961 -18.650 97.472 1.00 35.91
ATOM 809 O PRO 103 17.379 -17.803 96.789 1.00 35.91
ATOM 810 N SER 104 18.833 -19.516 96.958 1.00 42.91
ATOM 811 CA SER 104 19.119 -19.556 95.524 1.00 42.91
ATOM 812 CB SER 104 19.973 -20.773 95.166 1.00 41.87
ATOM 813 OG SER 104 21.321 -20.599 95.559 1.00 41.87

ATOM 814 C SER 104 17.779 -19.663 94.820 1.00 42.91
ATOM 815 O SER 104 16.993 -20.575 95.089 1.00 42.91
ATOM 816 N PRO 105 17.494 -18.735 93.905 1.00 35.96
ATOM 817 CD PRO 105 18.214 -17.490 93.571 1.00 31.16
ATOM 818 CA PRO 105 16.195 -18.829 93.228 1.00 35.96
ATOM 819 CB PRO 105 16.084 -17.493 92.488 1.00 31.16
ATOM 820 CG PRO 105 17.542 -17.062 92.303 1.00 31.16
ATOM 821 C PRO 105 15.989 -20.037 92.322 1.00 35.96
ATOM 822 O PRO 105 16.929 -20.569 91.726 1.00 35.96
ATOM 823 N THR 106 14.740 -20.472 92.249 1.00 50.79
ATOM 824 CA THR 106 14.354 -21.596 91.410 1.00 50.79
ATOM 825 CB THR 106 13.050 -22.237 91.919 1.00 55.41
ATOM 826 OG1 THR 106 11.965 -21.318 91.742 1.00 55.41
ATOM 827 CG2 THR 106 13.166 -22.582 93.399 1.00 55.41
ATOM 828 C THR 106 14.110 -21.029 90.015 1.00 50.79
ATOM 829 O THR 106 13.845 -19.833 89.865 1.00 50.79
ATOM 830 N THR 107 14.187 -21.871 88.990 1.00 61.13
ATOM 831 CA THR 107 13.973 -21.366 87.643 1.00 61.13
ATOM 832 CB THR 107 14.421 -22.403 86.562 1.00 71.51
ATOM 833 OG1 THR 107 15.405 -21.800 85.707 1.00 71.51
ATOM 834 CG2 THR 107 13.244 -22.863 85.713 1.00 71.51
ATOM 835 C THR 107 12.515 -20.960 87.454 1.00 61.13
ATOM 836 O THR 107 12.166 -20.321 86.471 1.00 61.13
ATOM 837 N GLU 108 11.663 -21.311 88.405 1.00 63.04

ATOM	838	CA	GLU	108	10.262	-20.940	88.297	1.00 63.04
ATOM	839	CB	GLU	108	9.377	-21.956	89.023	1.00 93.70
ATOM	840	CG	GLU	108	8.021	-22.149	88.359	1.00 93.70
ATOM	841	CD	GLU	108	7.250	-23.329	88.922	1.00 93.70
ATOM	842	OE1	GLU	108	6.636	-23.184	90.001	1.00 93.70
ATOM	843	OE2	GLU	108	7.266	-24.406	88.286	1.00 93.70
ATOM	844	C	GLU	108	10.077	-19.544	88.891	1.00 63.04
ATOM	845	O	GLU	108	9.144	-18.822	88.526	1.00 63.04
ATOM	846	N	GLN	109	10.968	-19.173	89.808	1.00 42.84
ATOM	847	CA	GLN	109	10.924	-17.854	90.429	1.00 42.84
ATOM	848	CB	GLN	109	11.763	-17.824	91.710	1.00 42.35
ATOM	849	CG	GLN	109	11.132	-18.557	92.890	1.00 42.35
ATOM	850	CD	GLN	109	11.971	-18.472	94.156	1.00 42.35
ATOM	851	OE1	GLN	109	13.094	-18.964	94.204	1.00 42.35
ATOM	852	NE2	GLN	109	11.423	-17.842	95.186	1.00 42.35
ATOM	853	C	GLN	109	11.473	-16.836	89.434	1.00 42.84
ATOM	854	O	GLN	109	10.866	-15.794	89.200	1.00 42.84
ATOM	855	N	ARG	110	12.624	-17.148	88.846	1.00 37.85
ATOM	856	CA	ARG	110	13.237	-16.258	87.873	1.00 37.85
ATOM	857	CB	ARG	110	14.464	-16.909	87.244	1.00 51.08
ATOM	858	CG	ARG	110	15.598	-17.191	88.202	1.00 51.08
ATOM	859	CD	ARG	110	16.692	-17.973	87.495	1.00 51.08
ATOM	860	NE	ARG	110	17.755	-18.390	88.405	1.00 51.08
ATOM	861	CZ	ARG	110	18.663	-17.569	88.927	1.00 51.08

ATOM	862	NH1	ARG	110	18.644	-16.275	88.629	1.00 51.08
ATOM	863	NH2	ARG	110	19.592	-18.042	89.751	1.00 51.08
ATOM	864	C	ARG	110	12.224	-15.946	86.783	1.00 37.85
ATOM	865	O	ARG	110	11.986	-14.782	86.457	1.00 37.85
ATOM	866	N	ALA	111	11.623	-16.992	86.224	1.00 49.18
ATOM	867	CA	ALA	111	10.634	-16.817	85.168	1.00 49.18
ATOM	868	CB	ALA	111	10.130	-18.172	84.682	1.00 35.40
ATOM	869	C	ALA	111	9.473	-15.978	85.689	1.00 49.18
ATOM	870	O	ALA	111	8.913	-15.158	84.961	1.00 49.18
ATOM	871	N	LYS	112	9.119	-16.175	86.954	1.00 39.11
ATOM	872	CA	LYS	112	8.023	-15.416	87.545	1.00 39.11
ATOM	873	CB	LYS	112	7.687	-15.945	88.940	1.00 85.43
ATOM	874	CG	LYS	112	6.573	-15.162	89.618	1.00 85.43
ATOM	875	CD	LYS	112	6.468	-15.473	91.105	1.00 85.43
ATOM	876	CE	LYS	112	5.400	-14.606	91.768	1.00 85.43
ATOM	877	NZ	LYS	112	5.303	-14.821	93.242	1.00 85.43
ATOM	878	C	LYS	112	8.380	-13.936	87.642	1.00 39.11
ATOM	879	O	LYS	112	7.586	-13.076	87.268	1.00 39.11
ATOM	880	N	TRP	113	9.578	-13.649	88.146	1.00 31.83
ATOM	881	CA	TRP	113	10.042	-12.274	88.299	1.00 31.83
ATOM	882	CB	TRP	113	11.368	-12.237	89.075	1.00 25.54
ATOM	883	CG	TRP	113	11.256	-12.810	90.459	1.00 25.54
ATOM	884	CD2	TRP	113	12.278	-13.490	91.203	1.00 25.54
ATOM	885	CE2	TRP	113	11.711	-13.879	92.434	1.00 25.54

ATOM 886 CE3 TRP 113 13.618 -13.816 90.945 1.00 25.54
ATOM 887 CD1 TRP 113 10.149 -12.803 91.253 1.00 25.54
ATOM 888 NE1 TRP 113 10.411 -13.443 92.439 1.00 25.54
ATOM 889 CZ2 TRP 113 12.434 -14.574 93.412 1.00 25.54
ATOM 890 CZ3 TRP 113 14.342 -14.510 91.927 1.00 25.54
ATOM 891 CH2 TRP 113 13.744 -14.881 93.140 1.00 25.54
ATOM 892 C TRP 113 10.202 -11.542 86.966 1.00 31.83
ATOM 893 O TRP 113 9.883 -10.362 86.868 1.00 31.83
ATOM 894 N VAL 114 10.694 -12.235 85.944 1.00 37.08
ATOM 895 CA VAL 114 10.873 -11.619 84.631 1.00 37.08
ATOM 896 CB VAL 114 11.568 -12.588 83.636 1.00 25.80
ATOM 897 CG1 VAL 114 11.544 -12.001 82.229 1.00 25.80
ATOM 898 CG2 VAL 114 13.006 -12.844 84.067 1.00 25.80
ATOM 899 C VAL 114 9.504 -11.247 84.078 1.00 37.08
ATOM 900 O VAL 114 9.323 -10.198 83.456 1.00 37.08
ATOM 901 N LYS 115 8.540 -12.122 84.314 1.00 37.76
ATOM 902 CA LYS 115 7.177 -11.911 83.861 1.00 37.76
ATOM 903 CB LYS 115 6.345 -13.151 84.189 1.00 60.43
ATOM 904 CG LYS 115 5.206 -13.437 83.232 1.00 60.43
ATOM 905 CD LYS 115 3.844 -13.292 83.904 1.00 60.43
ATOM 906 CE LYS 115 2.935 -14.479 83.591 1.00 60.43
ATOM 907 NZ LYS 115 1.512 -14.209 83.953 1.00 60.43
ATOM 908 C LYS 115 6.613 -10.682 84.580 1.00 37.76
ATOM 909 O LYS 115 5.863 -9.895 83.999 1.00 37.76

ATOM	910	N	LYS	116	6.988	-10.519	85.847	1.00	44.68
ATOM	911	CA	LYS	116	6.528	-9.392	86.649	1.00	44.68
ATOM	912	CB	LYS	116	6.848	-9.639	88.124	1.00	47.83
ATOM	913	CG	LYS	116	6.427	-8.510	89.052	1.00	47.83
ATOM	914	CD	LYS	116	6.846	-8.804	90.480	1.00	47.83
ATOM	915	CE	LYS	116	6.582	-7.623	91.400	1.00	47.83
ATOM	916	NZ	LYS	116	7.136	-7.862	92.768	1.00	47.83
ATOM	917	C	LYS	116	7.174	-8.081	86.184	1.00	44.68
ATOM	918	O	LYS	116	6.527	-7.029	86.162	1.00	44.68
ATOM	919	N	ALA	117	8.450	-8.142	85.816	1.00	31.99
ATOM	920	CA	ALA	117	9.152	-6.959	85.345	1.00	31.99
ATOM	921	CB	ALA	117	10.615	-7.272	85.121	1.00	21.76
ATOM	922	C	ALA	117	8.525	-6.491	84.043	1.00	31.99
ATOM	923	O	ALA	117	8.311	-5.302	83.832	1.00	31.99
ATOM	924	N	LYS	118	8.222	-7.443	83.173	1.00	40.71
ATOM	925	CA	LYS	118	7.632	-7.137	81.883	1.00	40.71
ATOM	926	CB	LYS	118	7.499	-8.421	81.063	1.00	61.77
ATOM	927	CG	LYS	118	7.281	-8.200	79.577	1.00	61.77
ATOM	928	CD	LYS	118	7.510	-9.495	78.812	1.00	61.77
ATOM	929	CE	LYS	118	7.974	-9.237	77.381	1.00	61.77
ATOM	930	NZ	LYS	118	8.495	-10.490	76.749	1.00	61.77
ATOM	931	C	LYS	118	6.271	-6.474	82.027	1.00	40.71
ATOM	932	O	LYS	118	5.953	-5.529	81.312	1.00	40.71
ATOM	933	N	GLU	119	5.462	-6.956	82.956	1.00	33.86

ATOM	934	CA	GLU	119	4.140	-6.377	83.111	1.00	33.86
ATOM	935	CB	GLU	119	3.274	-7.279	83.991	1.00	64.63
ATOM	936	CG	GLU	119	1.788	-7.224	83.639	1.00	64.63
ATOM	937	CD	GLU	119	1.530	-7.160	82.133	1.00	64.63
ATOM	938	OE1	GLU	119	0.818	-6.227	81.703	1.00	64.63
ATOM	939	OE2	GLU	119	2.032	-8.028	81.381	1.00	64.63
ATOM	940	C	GLU	119	4.245	-4.962	83.674	1.00	33.86
ATOM	941	O	GLU	119	3.429	-4.095	83.362	1.00	33.86
ATOM	942	N	ILE	120	5.270	-4.730	84.487	1.00	30.00
ATOM	943	CA	ILE	120	5.500	-3.420	85.063	1.00	30.00
ATOM	944	CB	ILE	120	6.656	-3.458	86.100	1.00	30.15
ATOM	945	CG2	ILE	120	7.168	-2.049	86.372	1.00	30.15
ATOM	946	CG1	ILE	120	6.176	-4.116	87.398	1.00	30.15
ATOM	947	CD1	ILE	120	7.292	-4.435	88.385	1.00	30.15
ATOM	948	C	ILE	120	5.882	-2.461	83.935	1.00	30.00
ATOM	949	O	ILE	120	5.408	-1.325	83.882	1.00	30.00
ATOM	950	N	VAL	121	6.741	-2.927	83.035	1.00	35.95
ATOM	951	CA	VAL	121	7.188	-2.105	81.923	1.00	35.95
ATOM	952	CB	VAL	121	8.459	-2.707	81.278	1.00	26.65
ATOM	953	CG1	VAL	121	8.765	-2.014	79.968	1.00	26.65
ATOM	954	CG2	VAL	121	9.636	-2.547	82.230	1.00	26.65
ATOM	955	C	VAL	121	6.094	-1.913	80.869	1.00	35.95
ATOM	956	O	VAL	121	6.014	-0.854	80.230	1.00	35.95
ATOM	957	N	GLU	122	5.261	-2.934	80.674	1.00	34.93

ATOM	958	CA	GLU	122	4.169	-2.822	79.718	1.00	34.93
ATOM	959	CB	GLU	122	3.357	-4.114	79.647	1.00	40.08
ATOM	960	CG	GLU	122	3.868	-5.140	78.652	1.00	40.08
ATOM	961	CD	GLU	122	3.852	-4.634	77.226	1.00	40.08
ATOM	962	OE1	GLU	122	4.946	-4.398	76.666	1.00	40.08
ATOM	963	OE2	GLU	122	2.747	-4.469	76.667	1.00	40.08
ATOM	964	C	GLU	122	3.260	-1.697	80.191	1.00	34.93
ATOM	965	O	GLU	122	2.807	-0.877	79.397	1.00	34.93
ATOM	966	N	GLU	123	2.992	-1.665	81.491	1.00	32.96
ATOM	967	CA	GLU	123	2.138	-0.630	82.043	1.00	32.96
ATOM	968	CB	GLU	123	1.928	-0.843	83.547	1.00	59.85
ATOM	969	CG	GLU	123	0.992	-1.999	83.912	1.00	59.85
ATOM	970	CD	GLU	123	-0.405	-1.850	83.318	1.00	59.85
ATOM	971	OE1	GLU	123	-0.950	-0.726	83.334	1.00	59.85
ATOM	972	OE2	GLU	123	-0.970	-2.860	82.843	1.00	59.85
ATOM	973	C	GLU	123	2.732	0.753	81.785	1.00	32.96
ATOM	974	O	GLU	123	2.006	1.695	81.462	1.00	32.96
ATOM	975	N	GLY	124	4.052	0.870	81.920	1.00	39.02
ATOM	976	CA	GLY	124	4.707	2.147	81.690	1.00	39.02
ATOM	977	C	GLY	124	4.549	2.599	80.249	1.00	39.02
ATOM	978	O	GLY	124	4.365	3.783	79.972	1.00	39.02
ATOM	979	N	LYS	125	4.620	1.643	79.328	1.00	35.24
ATOM	980	CA	LYS	125	4.474	1.925	77.909	1.00	35.24
ATOM	981	CB	LYS	125	4.620	0.661	77.098	1.00	34.74

ATOM 982 CG LYS 125 5.913 -0.048 77.285 1.00 34.74
ATOM 983 CD LYS 125 5.958 -1.196 76.321 1.00 34.74
ATOM 984 CE LYS 125 7.363 -1.576 76.003 1.00 34.74
ATOM 985 NZ LYS 125 7.313 -2.617 74.961 1.00 34.74
ATOM 986 C LYS 125 3.097 2.466 77.638 1.00 35.24
ATOM 987 O LYS 125 2.926 3.440 76.893 1.00 35.24
ATOM 988 N ILE 126 2.104 1.794 78.215 1.00 32.74
ATOM 989 CA ILE 126 0.725 2.208 78.047 1.00 32.74
ATOM 990 CB ILE 126 -0.219 1.320 78.864 1.00 29.38
ATOM 991 CG2 ILE 126 -1.642 1.859 78.801 1.00 29.38
ATOM 992 CG1 ILE 126 -0.154 -0.112 78.335 1.00 29.38
ATOM 993 CD1 ILE 126 -1.019 -1.088 79.110 1.00 29.38
ATOM 994 C ILE 126 0.628 3.650 78.522 1.00 32.74
ATOM 995 O ILE 126 -0.019 4.475 77.885 1.00 32.74
ATOM 996 N ALA 127 1.302 3.956 79.626 1.00 29.45
ATOM 997 CA ALA 127 1.284 5.309 80.166 1.00 29.45
ATOM 998 CB ALA 127 2.028 5.356 81.501 1.00 24.11
ATOM 999 C ALA 127 1.922 6.273 79.170 1.00 29.45
ATOM 1000 O ALA 127 1.434 7.386 78.978 1.00 29.45
ATOM 1001 N ILE 128 3.013 5.847 78.536 1.00 32.93
ATOM 1002 CA ILE 128 3.691 6.687 77.552 1.00 32.93
ATOM 1003 CB ILE 128 4.936 5.983 76.957 1.00 23.94
ATOM 1004 CG2 ILE 128 5.442 6.742 75.725 1.00 23.94
ATOM 1005 CG1 ILE 128 6.031 5.871 78.021 1.00 23.94

ATOM	1006	CD1	ILE	128	6.454	7.190	78.612	1.00	23.94
ATOM	1007	C	ILE	128	2.732	7.015	76.413	1.00	32.93
ATOM	1008	O	ILE	128	2.524	8.173	76.077	1.00	32.93
ATOM	1009	N	ARG	129	2.142	5.985	75.826	1.00	31.09
ATOM	1010	CA	ARG	129	1.221	6.190	74.731	1.00	31.09
ATOM	1011	CB	ARG	129	0.764	4.844	74.183	1.00	31.49
ATOM	1012	CG	ARG	129	1.917	3.977	73.731	1.00	31.49
ATOM	1013	CD	ARG	129	1.471	2.930	72.738	1.00	31.49
ATOM	1014	NE	ARG	129	2.600	2.124	72.292	1.00	31.49
ATOM	1015	CZ	ARG	129	2.977	0.984	72.858	1.00	31.49
ATOM	1016	NH1	ARG	129	2.300	0.510	73.894	1.00	31.49
ATOM	1017	NH2	ARG	129	4.040	0.326	72.396	1.00	31.49
ATOM	1018	C	ARG	129	0.016	7.040	75.110	1.00	31.09
ATOM	1019	O	ARG	129	-0.590	7.665	74.242	1.00	31.09
ATOM	1020	N	ASN	130	-0.348	7.069	76.389	1.00	30.83
ATOM	1021	CA	ASN	130	-1.489	7.881	76.786	1.00	30.83
ATOM	1022	CB	ASN	130	-1.981	7.517	78.193	1.00	39.26
ATOM	1023	CG	ASN	130	-2.935	6.328	78.185	1.00	39.26
ATOM	1024	OD1	ASN	130	-3.616	6.075	77.188	1.00	39.26
ATOM	1025	ND2	ASN	130	-3.005	5.610	79.301	1.00	39.26
ATOM	1026	C	ASN	130	-1.083	9.340	76.732	1.00	30.83
ATOM	1027	O	ASN	130	-1.826	10.185	76.230	1.00	30.83
ATOM	1028	N	ILE	131	0.108	9.627	77.247	1.00	30.20
ATOM	1029	CA	ILE	131	0.635	10.981	77.229	1.00	30.20

ATOM 1030 CB ILE 131 2.061 11.008 77.807 1.00 27.12
ATOM 1031 CG2 ILE 131 2.740 12.339 77.485 1.00 27.12
ATOM 1032 CG1 ILE 131 2.003 10.728 79.310 1.00 27.12
ATOM 1033 CD1 ILE 131 3.357 10.540 79.955 1.00 27.12
ATOM 1034 C ILE 131 0.655 11.475 75.780 1.00 30.20
ATOM 1035 O ILE 131 0.271 12.607 75.493 1.00 30.20
ATOM 1036 N ARG 132 1.094 10.610 74.869 1.00 30.22
ATOM 1037 CA ARG 132 1.149 10.943 73.452 1.00 30.22
ATOM 1038 CB ARG 132 1.653 9.744 72.649 1.00 38.82
ATOM 1039 CG ARG 132 1.395 9.844 71.152 1.00 38.82
ATOM 1040 CD ARG 132 1.837 8.584 70.433 1.00 38.82
ATOM 1041 NE ARG 132 1.578 8.664 69.003 1.00 38.82
ATOM 1042 CZ ARG 132 0.370 8.586 68.441 1.00 38.82
ATOM 1043 NH1 ARG 132 -0.718 8.412 69.185 1.00 38.82
ATOM 1044 NH2 ARG 132 0.245 8.714 67.125 1.00 38.82
ATOM 1045 C ARG 132 -0.221 11.359 72.926 1.00 30.22
ATOM 1046 O ARG 132 -0.356 12.389 72.271 1.00 30.22
ATOM 1047 N ARG 133 -1.236 10.549 73.213 1.00 41.63
ATOM 1048 CA ARG 133 -2.593 10.829 72.757 1.00 41.63
ATOM 1049 CB ARG 133 -3.498 9.635 73.059 1.00 50.13
ATOM 1050 CG ARG 133 -4.978 9.972 73.139 1.00 50.13
ATOM 1051 CD ARG 133 -5.837 8.757 72.843 1.00 50.13
ATOM 1052 NE ARG 133 -5.188 7.531 73.287 1.00 50.13
ATOM 1053 CZ ARG 133 -5.005 7.195 74.560 1.00 50.13

ATOM 1054 NH1 ARG 133 -5.432 7.992 75.537 1.00 50.13
ATOM 1055 NH2 ARG 133 -4.373 6.066 74.854 1.00 50.13
ATOM 1056 C ARG 133 -3.209 12.105 73.328 1.00 41.63
ATOM 1057 O ARG 133 -3.878 12.844 72.611 1.00 41.63
ATOM 1058 N GLU 134 -2.989 12.366 74.612 1.00 37.67
ATOM 1059 CA GLU 134 -3.550 13.559 75.234 1.00 37.67
ATOM 1060 CB GLU 134 -3.257 13.580 76.738 1.00 95.55
ATOM 1061 CG GLU 134 -1.777 13.566 77.082 1.00 95.55
ATOM 1062 CD GLU 134 -1.425 14.478 78.245 1.00 95.55
ATOM 1063 OE1 GLU 134 -0.804 13.991 79.218 1.00 95.55
ATOM 1064 OE2 GLU 134 -1.763 15.682 78.179 1.00 95.55
ATOM 1065 C GLU 134 -2.996 14.826 74.594 1.00 37.67
ATOM 1066 O GLU 134 -3.747 15.736 74.254 1.00 37.67
ATOM 1067 N ILE 135 -1.678 14.874 74.431 1.00 35.92
ATOM 1068 CA ILE 135 -1.016 16.031 73.850 1.00 35.92
ATOM 1069 CB ILE 135 0.513 15.905 73.985 1.00 39.63
ATOM 1070 CG2 ILE 135 1.200 17.111 73.381 1.00 39.63
ATOM 1071 CG1 ILE 135 0.875 15.794 75.464 1.00 39.63
ATOM 1072 CD1 ILE 135 2.359 15.720 75.741 1.00 39.63
ATOM 1073 C ILE 135 -1.382 16.183 72.386 1.00 35.92
ATOM 1074 O ILE 135 -1.687 17.281 71.920 1.00 35.92
ATOM 1075 N LEU 136 -1.354 15.072 71.665 1.00 41.46
ATOM 1076 CA LEU 136 -1.682 15.081 70.254 1.00 41.46
ATOM 1077 CB LEU 136 -1.679 13.655 69.711 1.00 34.36

ATOM	1078	CG	LEU	136	-0.757	13.364	68.526	1.00 34.36
ATOM	1079	CD1	LEU	136	0.525	14.169	68.631	1.00 34.36
ATOM	1080	CD2	LEU	136	-0.460	11.869	68.494	1.00 34.36
ATOM	1081	C	LEU	136	-3.030	15.745	70.004	1.00 41.46
ATOM	1082	O	LEU	136	-3.172	16.506	69.056	1.00 41.46
ATOM	1083	N	LYS	137	-4.020	15.484	70.850	1.00 45.83
ATOM	1084	CA	LYS	137	-5.318	16.109	70.634	1.00 45.83
ATOM	1085	CB	LYS	137	-6.449	15.330	71.326	1.00 74.57
ATOM	1086	CG	LYS	137	-6.404	15.320	72.835	1.00 74.57
ATOM	1087	CD	LYS	137	-7.364	14.283	73.409	1.00 74.57
ATOM	1088	CE	LYS	137	-8.739	14.854	73.707	1.00 74.57
ATOM	1089	NZ	LYS	137	-9.587	13.839	74.405	1.00 74.57
ATOM	1090	C	LYS	137	-5.311	17.559	71.087	1.00 45.83
ATOM	1091	O	LYS	137	-6.112	18.355	70.611	1.00 45.83
ATOM	1092	N	LYS	138	-4.421	17.911	72.008	1.00 38.48
ATOM	1093	CA	LYS	138	-4.343	19.298	72.434	1.00 38.48
ATOM	1094	CB	LYS	138	-3.485	19.450	73.689	1.00 46.05
ATOM	1095	CG	LYS	138	-4.322	19.614	74.943	1.00 46.05
ATOM	1096	CD	LYS	138	-3.486	19.890	76.177	1.00 46.05
ATOM	1097	CE	LYS	138	-2.736	18.654	76.639	1.00 46.05
ATOM	1098	NZ	LYS	138	-2.026	18.906	77.934	1.00 46.05
ATOM	1099	C	LYS	138	-3.751	20.104	71.278	1.00 38.48
ATOM	1100	O	LYS	138	-4.104	21.266	71.070	1.00 38.48
ATOM	1101	N	ILE	139	-2.859	19.476	70.518	1.00 31.21

ATOM	1102	CA	ILE	139	-2.255	20.128	69.366	1.00 31.21
ATOM	1103	CB	ILE	139	-1.038	19.329	68.845	1.00 30.83
ATOM	1104	CG2	ILE	139	-0.604	19.859	67.490	1.00 30.83
ATOM	1105	CG1	ILE	139	0.124	19.430	69.840	1.00 30.83
ATOM	1106	CD1	ILE	139	1.240	18.448	69.571	1.00 30.83
ATOM	1107	C	ILE	139	-3.311	20.222	68.264	1.00 31.21
ATOM	1108	O	ILE	139	-3.432	21.249	67.600	1.00 31.21
ATOM	1109	N	LYS	140	-4.077	19.147	68.081	1.00 37.01
ATOM	1110	CA	LYS	140	-5.129	19.105	67.068	1.00 37.01
ATOM	1111	CB	LYS	140	-5.858	17.765	67.111	1.00 60.99
ATOM	1112	CG	LYS	140	-6.134	17.149	65.751	1.00 60.99
ATOM	1113	CD	LYS	140	-4.888	16.462	65.195	1.00 60.99
ATOM	1114	CE	LYS	140	-5.206	15.633	63.949	1.00 60.99
ATOM	1115	NZ	LYS	140	-4.049	14.793	63.505	1.00 60.99
ATOM	1116	C	LYS	140	-6.136	20.223	67.322	1.00 37.01
ATOM	1117	O	LYS	140	-6.665	20.828	66.383	1.00 37.01
ATOM	1118	N	GLU	141	-6.402	20.485	68.599	1.00 32.47
ATOM	1119	CA	GLU	141	-7.335	21.527	68.994	1.00 32.47
ATOM	1120	CB	GLU	141	-7.497	21.545	70.512	1.00 56.18
ATOM	1121	CG	GLU	141	-8.789	20.933	71.011	1.00 56.18
ATOM	1122	CD	GLU	141	-8.646	20.300	72.394	1.00 56.18
ATOM	1123	OE1	GLU	141	-8.088	20.951	73.310	1.00 56.18
ATOM	1124	OE2	GLU	141	-9.102	19.146	72.562	1.00 56.18
ATOM	1125	C	GLU	141	-6.797	22.862	68.527	1.00 32.47

ATOM 1126 O GLU 141 -7.491 23.630 67.860 1.00 32.47
ATOM 1127 N ASP 142 -5.548 23.135 68.887 1.00 43.49
ATOM 1128 CA ASP 142 -4.911 24.379 68.504 1.00 43.49
ATOM 1129 CB ASP 142 -3.510 24.449 69.105 1.00 40.36
ATOM 1130 CG ASP 142 -3.523 24.880 70.557 1.00 40.36
ATOM 1131 OD1 ASP 142 -4.599 24.852 71.180 1.00 40.36
ATOM 1132 OD2 ASP 142 -2.455 25.246 71.082 1.00 40.36
ATOM 1133 C ASP 142 -4.860 24.497 66.987 1.00 43.49
ATOM 1134 O ASP 142 -5.002 25.592 66.443 1.00 43.49
ATOM 1135 N GLN 143 -4.667 23.374 66.302 1.00 38.41
ATOM 1136 CA GLN 143 -4.634 23.401 64.848 1.00 38.41
ATOM 1137 CB GLN 143 -4.197 22.045 64.270 1.00 36.16
ATOM 1138 CG GLN 143 -4.584 21.868 62.799 1.00 36.16
ATOM 1139 CD GLN 143 -4.063 20.585 62.152 1.00 36.16
ATOM 1140 OE1 GLN 143 -3.863 19.562 62.814 1.00 36.16
ATOM 1141 NE2 GLN 143 -3.869 20.632 60.843 1.00 36.16
ATOM 1142 C GLN 143 -6.025 23.757 64.329 1.00 38.41
ATOM 1143 O GLN 143 -6.161 24.502 63.362 1.00 38.41
ATOM 1144 N LYS 144 -7.060 23.238 64.981 1.00 41.80
ATOM 1145 CA LYS 144 -8.428 23.511 64.549 1.00 41.80
ATOM 1146 CB LYS 144 -9.405 22.591 65.283 1.00 70.22
ATOM 1147 CG LYS 144 -10.807 22.637 64.711 1.00 70.22
ATOM 1148 CD LYS 144 -11.689 21.509 65.226 1.00 70.22
ATOM 1149 CE LYS 144 -13.040 21.518 64.514 1.00 70.22

ATOM	1150	NZ	LYS	144	-13.922	20.387	64.915	1.00	70.22
ATOM	1151	C	LYS	144	-8.840	24.982	64.730	1.00	41.80
ATOM	1152	O	LYS	144	-9.540	25.542	63.885	1.00	41.80
ATOM	1153	N	GLU	145	-8.404	25.616	65.814	1.00	32.59
ATOM	1154	CA	GLU	145	-8.753	27.020	66.029	1.00	32.59
ATOM	1155	CB	GLU	145	-8.406	27.480	67.435	1.00	54.82
ATOM	1156	CG	GLU	145	-8.937	26.652	68.554	1.00	54.82
ATOM	1157	CD	GLU	145	-8.574	27.273	69.878	1.00	54.82
ATOM	1158	OE1	GLU	145	-8.448	26.529	70.874	1.00	54.82
ATOM	1159	OE2	GLU	145	-8.418	28.515	69.915	1.00	54.82
ATOM	1160	C	GLU	145	-7.997	27.933	65.078	1.00	32.59
ATOM	1161	O	GLU	145	-8.346	29.108	64.938	1.00	32.59
ATOM	1162	N	GLY	146	-6.938	27.406	64.463	1.00	27.98
ATOM	1163	CA	GLY	146	-6.153	28.200	63.539	1.00	27.98
ATOM	1164	C	GLY	146	-4.874	28.738	64.154	1.00	27.98
ATOM	1165	O	GLY	146	-4.094	29.421	63.487	1.00	27.98
ATOM	1166	N	LEU	147	-4.654	28.437	65.429	1.00	31.04
ATOM	1167	CA	LEU	147	-3.451	28.896	66.108	1.00	31.04
ATOM	1168	CB	LEU	147	-3.506	28.528	67.590	1.00	33.77
ATOM	1169	CG	LEU	147	-4.561	29.240	68.439	1.00	33.77
ATOM	1170	CD1	LEU	147	-4.518	28.698	69.852	1.00	33.77
ATOM	1171	CD2	LEU	147	-4.293	30.738	68.437	1.00	33.77
ATOM	1172	C	LEU	147	-2.211	28.269	65.481	1.00	31.04
ATOM	1173	O	LEU	147	-1.216	28.947	65.227	1.00	31.04

ATOM 1174 N ILE 148 -2.285 26.965 65.239 1.00 38.27
ATOM 1175 CA ILE 148 -1.182 26.213 64.658 1.00 38.27
ATOM 1176 CB ILE 148 -0.917 24.931 65.461 1.00 29.04
ATOM 1177 CG2 ILE 148 0.141 24.102 64.778 1.00 29.04
ATOM 1178 CG1 ILE 148 -0.503 25.287 66.884 1.00 29.04
ATOM 1179 CD1 ILE 148 -0.470 24.104 67.811 1.00 29.04
ATOM 1180 C ILE 148 -1.517 25.811 63.236 1.00 38.27
ATOM 1181 O ILE 148 -2.359 24.936 63.021 1.00 38.27
ATOM 1182 N PRO 149 -0.872 26.446 62.243 1.00 34.71
ATOM 1183 CD PRO 149 0.123 27.528 62.327 1.00 39.35
ATOM 1184 CA PRO 149 -1.157 26.094 60.849 1.00 34.71
ATOM 1185 CB PRO 149 -0.294 27.079 60.053 1.00 39.35
ATOM 1186 CG PRO 149 0.821 27.421 60.993 1.00 39.35
ATOM 1187 C PRO 149 -0.806 24.633 60.592 1.00 34.71
ATOM 1188 O PRO 149 0.086 24.083 61.236 1.00 34.71
ATOM 1189 N GLU 150 -1.518 24.010 59.657 1.00 45.51
ATOM 1190 CA GLU 150 -1.319 22.602 59.315 1.00 45.51
ATOM 1191 CB GLU 150 -1.953 22.304 57.960 1.00 61.98
ATOM 1192 CG GLU 150 -1.981 20.829 57.631 1.00 61.98
ATOM 1193 CD GLU 150 -2.892 20.505 56.460 1.00 61.98
ATOM 1194 OE1 GLU 150 -2.978 19.313 56.086 1.00 61.98
ATOM 1195 OE2 GLU 150 -3.524 21.438 55.915 1.00 61.98
ATOM 1196 C GLU 150 0.127 22.106 59.316 1.00 45.51
ATOM 1197 O GLU 150 0.452 21.141 60.001 1.00 45.51

ATOM	1198	N	ASP	151	0.994	22.753	58.549	1.00	41.19
ATOM	1199	CA	ASP	151	2.392	22.340	58.486	1.00	41.19
ATOM	1200	CB	ASP	151	3.152	23.242	57.517	1.00	62.06
ATOM	1201	CG	ASP	151	2.710	23.042	56.088	1.00	62.06
ATOM	1202	OD1	ASP	151	3.050	23.886	55.232	1.00	62.06
ATOM	1203	OD2	ASP	151	2.025	22.033	55.817	1.00	62.06
ATOM	1204	C	ASP	151	3.096	22.322	59.845	1.00	41.19
ATOM	1205	O	ASP	151	3.879	21.416	60.131	1.00	41.19
ATOM	1206	N	ASP	152	2.825	23.316	60.683	1.00	40.51
ATOM	1207	CA	ASP	152	3.461	23.366	61.998	1.00	40.51
ATOM	1208	CB	ASP	152	3.259	24.736	62.642	1.00	57.97
ATOM	1209	CG	ASP	152	3.931	25.841	61.865	1.00	57.97
ATOM	1210	OD1	ASP	152	3.460	26.168	60.760	1.00	57.97
ATOM	1211	OD2	ASP	152	4.942	26.379	62.354	1.00	57.97
ATOM	1212	C	ASP	152	2.908	22.282	62.910	1.00	40.51
ATOM	1213	O	ASP	152	3.598	21.794	63.799	1.00	40.51
ATOM	1214	N	ALA	153	1.656	21.907	62.678	1.00	34.68
ATOM	1215	CA	ALA	153	1.012	20.881	63.472	1.00	34.68
ATOM	1216	CB	ALA	153	-0.485	20.867	63.185	1.00	29.15
ATOM	1217	C	ALA	153	1.633	19.532	63.130	1.00	34.68
ATOM	1218	O	ALA	153	1.885	18.707	64.010	1.00	34.68
ATOM	1219	N	LYS	154	1.895	19.314	61.848	1.00	39.97
ATOM	1220	CA	LYS	154	2.484	18.057	61.424	1.00	39.97
ATOM	1221	CB	LYS	154	2.539	17.971	59.899	1.00	79.16

ATOM 1222 CG LYS 154 1.176 17.724 59.270 1.00 79.16
ATOM 1223 CD LYS 154 1.238 17.768 57.753 1.00 79.16
ATOM 1224 CE LYS 154 -0.150 17.675 57.135 1.00 79.16
ATOM 1225 NZ LYS 154 -0.105 17.865 55.654 1.00 79.16
ATOM 1226 C LYS 154 3.861 17.813 62.014 1.00 39.97
ATOM 1227 O LYS 154 4.124 16.716 62.498 1.00 39.97
ATOM 1228 N ARG 155 4.740 18.813 62.005 1.00 39.62
ATOM 1229 CA ARG 155 6.075 18.588 62.560 1.00 39.62
ATOM 1230 CB ARG 155 7.053 19.698 62.160 1.00 60.09
ATOM 1231 CG ARG 155 6.945 20.956 62.975 1.00 60.09
ATOM 1232 CD ARG 155 8.256 21.723 62.990 1.00 60.09
ATOM 1233 NE ARG 155 8.047 23.093 63.448 1.00 60.09
ATOM 1234 CZ ARG 155 7.654 23.432 64.674 1.00 60.09
ATOM 1235 NH1 ARG 155 7.434 22.501 65.594 1.00 60.09
ATOM 1236 NH2 ARG 155 7.447 24.709 64.972 1.00 60.09
ATOM 1237 C ARG 155 6.028 18.462 64.081 1.00 39.62
ATOM 1238 O ARG 155 6.890 17.831 64.693 1.00 39.62
ATOM 1239 N LEU 156 5.014 19.067 64.686 1.00 37.71
ATOM 1240 CA LEU 156 4.840 19.002 66.131 1.00 37.71
ATOM 1241 CB LEU 156 3.717 19.951 66.556 1.00 41.73
ATOM 1242 CG LEU 156 3.959 20.955 67.691 1.00 41.73
ATOM 1243 CD1 LEU 156 5.447 21.194 67.929 1.00 41.73
ATOM 1244 CD2 LEU 156 3.258 22.257 67.324 1.00 41.73
ATOM 1245 C LEU 156 4.473 17.558 66.471 1.00 37.71

ATOM 1246 O LEU 156 4.996 16.982 67.420 1.00 37.71
ATOM 1247 N GLU 157 3.578 16.981 65.673 1.00 38.40
ATOM 1248 CA GLU 157 3.140 15.606 65.867 1.00 38.40
ATOM 1249 CB GLU 157 2.013 15.278 64.885 1.00 42.33
ATOM 1250 CG GLU 157 0.741 16.056 65.182 1.00 42.33
ATOM 1251 CD GLU 157 -0.137 16.281 63.961 1.00 42.33
ATOM 1252 OE1 GLU 157 -1.113 17.060 64.072 1.00 42.33
ATOM 1253 OE2 GLU 157 0.145 15.690 62.896 1.00 42.33
ATOM 1254 C GLU 157 4.312 14.644 65.687 1.00 38.40
ATOM 1255 O GLU 157 4.451 13.682 66.443 1.00 38.40
ATOM 1256 N ASN 158 5.157 14.912 64.694 1.00 42.65
ATOM 1257 CA ASN 158 6.323 14.072 64.437 1.00 42.65
ATOM 1258 CB ASN 158 7.060 14.549 63.187 1.00 53.78
ATOM 1259 CG ASN 158 6.273 14.298 61.923 1.00 53.78
ATOM 1260 OD1 ASN 158 6.565 14.868 60.868 1.00 53.78
ATOM 1261 ND2 ASN 158 5.268 13.432 62.016 1.00 53.78
ATOM 1262 C ASN 158 7.274 14.100 65.627 1.00 42.65
ATOM 1263 O ASN 158 7.824 13.068 66.021 1.00 42.65
ATOM 1264 N GLU 159 7.468 15.282 66.203 1.00 41.03
ATOM 1265 CA GLU 159 8.352 15.400 67.350 1.00 41.03
ATOM 1266 CB GLU 159 8.517 16.865 67.760 1.00 80.12
ATOM 1267 CG GLU 159 9.384 17.043 68.999 1.00 80.12
ATOM 1268 CD GLU 159 10.772 16.430 68.852 1.00 80.12
ATOM 1269 OE1 GLU 159 11.367 16.051 69.889 1.00 80.12

ATOM 1270 OE2 GLU 159 11.270 16.337 67.708 1.00 80.12
ATOM 1271 C GLU 159 7.803 14.579 68.516 1.00 41.03
ATOM 1272 O GLU 159 8.551 13.882 69.200 1.00 41.03
ATOM 1273 N ILE 160 6.496 14.656 68.737 1.00 37.16
ATOM 1274 CA ILE 160 5.878 13.896 69.810 1.00 37.16
ATOM 1275 CB ILE 160 4.369 14.254 69.961 1.00 41.43
ATOM 1276 CG2 ILE 160 3.548 13.011 70.247 1.00 41.43
ATOM 1277 CG1 ILE 160 4.173 15.224 71.130 1.00 41.43
ATOM 1278 CD1 ILE 160 4.867 16.538 70.976 1.00 41.43
ATOM 1279 C ILE 160 6.040 12.413 69.502 1.00 37.16
ATOM 1280 O ILE 160 6.320 11.607 70.386 1.00 37.16
ATOM 1281 N GLN 161 5.875 12.056 68.236 1.00 40.44
ATOM 1282 CA GLN 161 6.007 10.667 67.824 1.00 40.44
ATOM 1283 CB GLN 161 5.642 10.518 66.348 1.00 47.39
ATOM 1284 CG GLN 161 5.636 9.082 65.878 1.00 47.39
ATOM 1285 CD GLN 161 4.721 8.211 66.716 1.00 47.39
ATOM 1286 OE1 GLN 161 5.116 7.136 67.178 1.00 47.39
ATOM 1287 NE2 GLN 161 3.489 8.667 66.914 1.00 47.39
ATOM 1288 C GLN 161 7.435 10.171 68.064 1.00 40.44
ATOM 1289 O GLN 161 7.643 9.034 68.498 1.00 40.44
ATOM 1290 N ALA 162 8.416 11.029 67.785 1.00 32.94
ATOM 1291 CA ALA 162 9.810 10.670 67.986 1.00 32.94
ATOM 1292 CB ALA 162 10.719 11.759 67.434 1.00 30.93
ATOM 1293 C ALA 162 10.056 10.465 69.478 1.00 32.94

ATOM 1294 O ALA 162 10.733 9.511 69.876 1.00 32.94
ATOM 1295 N LEU 163 9.492 11.350 70.299 1.00 31.29
ATOM 1296 CA LEU 163 9.629 11.256 71.759 1.00 31.29
ATOM 1297 CB LEU 163 8.952 12.438 72.446 1.00 44.08
ATOM 1298 CG LEU 163 9.756 13.730 72.562 1.00 44.08
ATOM 1299 CD1 LEU 163 8.899 14.794 73.242 1.00 44.08
ATOM 1300 CD2 LEU 163 11.026 13.467 73.362 1.00 44.08
ATOM 1301 C LEU 163 9.016 9.970 72.298 1.00 31.29
ATOM 1302 O LEU 163 9.566 9.337 73.197 1.00 31.29
ATOM 1303 N THR 164 7.867 9.601 71.746 1.00 30.16
ATOM 1304 CA THR 164 7.175 8.389 72.147 1.00 30.16
ATOM 1305 CB THR 164 5.812 8.280 71.431 1.00 32.81
ATOM 1306 OG1 THR 164 4.972 9.360 71.849 1.00 32.81
ATOM 1307 CG2 THR 164 5.125 6.972 71.766 1.00 32.81
ATOM 1308 C THR 164 8.033 7.163 71.825 1.00 30.16
ATOM 1309 O THR 164 8.212 6.290 72.674 1.00 30.16
ATOM 1310 N ASP 165 8.567 7.093 70.609 1.00 36.74
ATOM 1311 CA ASP 165 9.405 5.954 70.237 1.00 36.74
ATOM 1312 CB ASP 165 9.809 6.009 68.764 1.00 43.20
ATOM 1313 CG ASP 165 8.627 6.038 67.838 1.00 43.20
ATOM 1314 OD1 ASP 165 7.598 5.410 68.157 1.00 43.20
ATOM 1315 OD2 ASP 165 8.733 6.683 66.781 1.00 43.20
ATOM 1316 C ASP 165 10.669 5.957 71.081 1.00 36.74
ATOM 1317 O ASP 165 11.175 4.910 71.468 1.00 36.74

ATOM 1318 N GLU 166 11.180 7.148 71.360 1.00 35.71
ATOM 1319 CA GLU 166 12.379 7.271 72.159 1.00 35.71
ATOM 1320 CB GLU 166 12.782 8.744 72.247 1.00 54.57
ATOM 1321 CG GLU 166 13.811 9.057 73.318 1.00 54.57
ATOM 1322 CD GLU 166 14.165 10.542 73.381 1.00 54.57
ATOM 1323 OE1 GLU 166 14.607 11.010 74.458 1.00 54.57
ATOM 1324 OE2 GLU 166 14.010 11.233 72.346 1.00 54.57
ATOM 1325 C GLU 166 12.131 6.678 73.549 1.00 35.71
ATOM 1326 O GLU 166 12.978 5.958 74.083 1.00 35.71
ATOM 1327 N PHE 167 10.966 6.957 74.128 1.00 32.84
ATOM 1328 CA PHE 167 10.664 6.437 75.455 1.00 32.84
ATOM 1329 CB PHE 167 9.675 7.349 76.181 1.00 33.04
ATOM 1330 CG PHE 167 10.337 8.524 76.824 1.00 33.04
ATOM 1331 CD1 PHE 167 10.602 9.677 76.093 1.00 33.04
ATOM 1332 CD2 PHE 167 10.806 8.435 78.127 1.00 33.04
ATOM 1333 CE1 PHE 167 11.330 10.732 76.650 1.00 33.04
ATOM 1334 CE2 PHE 167 11.536 9.483 78.695 1.00 33.04
ATOM 1335 CZ PHE 167 11.802 10.633 77.949 1.00 33.04
ATOM 1336 C PHE 167 10.205 4.992 75.492 1.00 32.84
ATOM 1337 O PHE 167 10.456 4.295 76.471 1.00 32.84
ATOM 1338 N ILE 168 9.534 4.536 74.439 1.00 29.66
ATOM 1339 CA ILE 168 9.129 3.141 74.378 1.00 29.66
ATOM 1340 CB ILE 168 8.321 2.835 73.090 1.00 23.21
ATOM 1341 CG2 ILE 168 8.154 1.336 72.914 1.00 23.21

ATOM 1342 CG1 ILE 168 6.956 3.527 73.151 1.00 23.21
ATOM 1343 CD1 ILE 168 6.045 3.006 74.261 1.00 23.21
ATOM 1344 C ILE 168 10.441 2.357 74.333 1.00 29.66
ATOM 1345 O ILE 168 10.576 1.314 74.963 1.00 29.66
ATOM 1346 N GLU 169 11.409 2.876 73.584 1.00 31.65
ATOM 1347 CA GLU 169 12.714 2.233 73.460 1.00 31.65
ATOM 1348 CB GLU 169 13.622 3.015 72.512 1.00 93.52
ATOM 1349 CG GLU 169 13.972 2.279 71.237 1.00 93.52
ATOM 1350 CD GLU 169 15.263 2.784 70.617 1.00 93.52
ATOM 1351 OE1 GLU 169 16.259 2.029 70.623 1.00 93.52
ATOM 1352 OE2 GLU 169 15.287 3.936 70.133 1.00 93.52
ATOM 1353 C GLU 169 13.399 2.133 74.811 1.00 31.65
ATOM 1354 O GLU 169 13.927 1.084 75.166 1.00 31.65
ATOM 1355 N LYS 170 13.397 3.233 75.557 1.00 29.27
ATOM 1356 CA LYS 170 14.020 3.262 76.869 1.00 29.27
ATOM 1357 CB LYS 170 13.933 4.665 77.479 1.00 37.44
ATOM 1358 CG LYS 170 14.604 5.778 76.681 1.00 37.44
ATOM 1359 CD LYS 170 14.878 6.964 77.592 1.00 37.44
ATOM 1360 CE LYS 170 15.005 8.291 76.849 1.00 37.44
ATOM 1361 NZ LYS 170 16.177 8.363 75.931 1.00 37.44
ATOM 1362 C LYS 170 13.345 2.258 77.799 1.00 29.27
ATOM 1363 O LYS 170 13.999 1.647 78.646 1.00 29.27
ATOM 1364 N LEU 171 12.034 2.090 77.651 1.00 41.19
ATOM 1365 CA LEU 171 11.309 1.134 78.481 1.00 41.19

ATOM 1366 CB LEU 171 9.802 1.224 78.237 1.00 27.02
ATOM 1367 CG LEU 171 8.903 1.952 79.251 1.00 27.02
ATOM 1368 CD1 LEU 171 9.643 2.164 80.555 1.00 27.02
ATOM 1369 CD2 LEU 171 8.446 3.275 78.696 1.00 27.02
ATOM 1370 C LEU 171 11.789 -0.271 78.153 1.00 41.19
ATOM 1371 O LEU 171 12.092 -1.059 79.048 1.00 41.19
ATOM 1372 N ASP 172 11.872 -0.577 76.863 1.00 35.05
ATOM 1373 CA ASP 172 12.321 -1.890 76.435 1.00 35.05
ATOM 1374 CB ASP 172 12.324 -2.001 74.907 1.00 41.75
ATOM 1375 CG ASP 172 10.923 -2.075 74.324 1.00 41.75
ATOM 1376 OD1 ASP 172 10.790 -2.135 73.080 1.00 41.75
ATOM 1377 OD2 ASP 172 9.954 -2.077 75.111 1.00 41.75
ATOM 1378 C ASP 172 13.711 -2.152 76.962 1.00 35.05
ATOM 1379 O ASP 172 14.037 -3.283 77.336 1.00 35.05
ATOM 1380 N GLU 173 14.533 -1.110 77.003 1.00 32.93
ATOM 1381 CA GLU 173 15.892 -1.278 77.491 1.00 32.93
ATOM 1382 CB GLU 173 16.706 -0.007 77.306 1.00 41.86
ATOM 1383 CG GLU 173 18.142 -0.213 77.734 1.00 41.86
ATOM 1384 CD GLU 173 18.999 1.008 77.563 1.00 41.86
ATOM 1385 OE1 GLU 173 18.671 1.847 76.688 1.00 41.86
ATOM 1386 OE2 GLU 173 20.009 1.112 78.294 1.00 41.86
ATOM 1387 C GLU 173 15.873 -1.630 78.965 1.00 32.93
ATOM 1388 O GLU 173 16.495 -2.601 79.400 1.00 32.93
ATOM 1389 N VAL 174 15.160 -0.811 79.726 1.00 31.38

ATOM 1390 CA VAL 174 15.023 -0.990 81.156 1.00 31.38
ATOM 1391 CB VAL 174 14.087 0.108 81.715 1.00 34.37
ATOM 1392 CG1 VAL 174 13.572 -0.277 83.053 1.00 34.37
ATOM 1393 CG2 VAL 174 14.846 1.426 81.815 1.00 34.37
ATOM 1394 C VAL 174 14.506 -2.401 81.488 1.00 31.38
ATOM 1395 O VAL 174 14.896 -2.997 82.492 1.00 31.38
ATOM 1396 N PHE 175 13.631 -2.943 80.648 1.00 32.64
ATOM 1397 CA PHE 175 13.136 -4.290 80.895 1.00 32.64
ATOM 1398 CB PHE 175 11.999 -4.661 79.947 1.00 30.70
ATOM 1399 CG PHE 175 11.718 -6.133 79.920 1.00 30.70
ATOM 1400 CD1 PHE 175 11.187 -6.770 81.038 1.00 30.70
ATOM 1401 CD2 PHE 175 12.056 -6.898 78.810 1.00 30.70
ATOM 1402 CE1 PHE 175 11.001 -8.159 81.056 1.00 30.70
ATOM 1403 CE2 PHE 175 11.878 -8.280 78.811 1.00 30.70
ATOM 1404 CZ PHE 175 11.348 -8.916 79.938 1.00 30.70
ATOM 1405 C PHE 175 14.262 -5.296 80.695 1.00 32.64
ATOM 1406 O PHE 175 14.444 -6.196 81.509 1.00 32.64
ATOM 1407 N GLU 176 15.002 -5.150 79.597 1.00 36.36
ATOM 1408 CA GLU 176 16.120 -6.039 79.290 1.00 36.36
ATOM 1409 CB GLU 176 16.826 -5.595 78.011 1.00 49.35
ATOM 1410 CG GLU 176 16.862 -6.649 76.921 1.00 49.35
ATOM 1411 CD GLU 176 17.554 -7.935 77.348 1.00 49.35
ATOM 1412 OE1 GLU 176 18.722 -7.869 77.793 1.00 49.35
ATOM 1413 OE2 GLU 176 16.929 -9.015 77.232 1.00 49.35

ATOM	1414	C	GLU	176	17.125	-6.050	80.428	1.00	36.36
ATOM	1415	O	GLU	176	17.603	-7.110	80.832	1.00	36.36
ATOM	1416	N	ILE	177	17.444	-4.866	80.942	1.00	35.83
ATOM	1417	CA	ILE	177	18.398	-4.740	82.033	1.00	35.83
ATOM	1418	CB	ILE	177	18.606	-3.259	82.422	1.00	29.26
ATOM	1419	CG2	ILE	177	19.370	-3.163	83.737	1.00	29.26
ATOM	1420	CG1	ILE	177	19.358	-2.525	81.303	1.00	29.26
ATOM	1421	CD1	ILE	177	20.851	-2.863	81.233	1.00	29.26
ATOM	1422	C	ILE	177	17.936	-5.513	83.265	1.00	35.83
ATOM	1423	O	ILE	177	18.738	-6.162	83.946	1.00	35.83
ATOM	1424	N	LYS	178	16.643	-5.448	83.554	1.00	34.13
ATOM	1425	CA	LYS	178	16.112	-6.147	84.707	1.00	34.13
ATOM	1426	CB	LYS	178	14.687	-5.684	85.003	1.00	28.12
ATOM	1427	CG	LYS	178	14.188	-6.109	86.376	1.00	28.12
ATOM	1428	CD	LYS	178	15.059	-5.482	87.469	1.00	28.12
ATOM	1429	CE	LYS	178	14.588	-5.848	88.857	1.00	28.12
ATOM	1430	NZ	LYS	178	15.375	-5.118	89.878	1.00	28.12
ATOM	1431	C	LYS	178	16.117	-7.645	84.444	1.00	34.13
ATOM	1432	O	LYS	178	16.470	-8.440	85.325	1.00	34.13
ATOM	1433	N	LYS	179	15.719	-8.028	83.232	1.00	34.54
ATOM	1434	CA	LYS	179	15.682	-9.435	82.865	1.00	34.54
ATOM	1435	CB	LYS	179	15.233	-9.610	81.419	1.00	32.56
ATOM	1436	CG	LYS	179	15.201	-11.056	80.963	1.00	32.56
ATOM	1437	CD	LYS	179	14.889	-11.124	79.487	1.00	32.56

ATOM 1438 CE LYS 179 14.908 -12.551 78.949 1.00 32.56
ATOM 1439 NZ LYS 179 14.654 -12.572 77.478 1.00 32.56
ATOM 1440 C LYS 179 17.064 -10.035 83.044 1.00 34.54
ATOM 1441 O LYS 179 17.202 -11.146 83.554 1.00 34.54
ATOM 1442 N GLU 180 18.085 -9.297 82.623 1.00 32.37
ATOM 1443 CA GLU 180 19.455 -9.765 82.765 1.00 32.37
ATOM 1444 CB GLU 180 20.427 -8.792 82.106 1.00 44.31
ATOM 1445 CG GLU 180 20.288 -8.756 80.608 1.00 44.31
ATOM 1446 CD GLU 180 21.035 -7.609 79.980 1.00 44.31
ATOM 1447 OE1 GLU 180 20.809 -7.366 78.779 1.00 44.31
ATOM 1448 OE2 GLU 180 21.841 -6.951 80.677 1.00 44.31
ATOM 1449 C GLU 180 19.793 -9.908 84.232 1.00 32.37
ATOM 1450 O GLU 180 20.371 -10.898 84.635 1.00 32.37
ATOM 1451 N GLU 181 19.422 -8.920 85.035 1.00 35.90
ATOM 1452 CA GLU 181 19.708 -8.966 86.465 1.00 35.90
ATOM 1453 CB GLU 181 19.124 -7.738 87.160 1.00 47.46
ATOM 1454 CG GLU 181 19.444 -7.662 88.642 1.00 47.46
ATOM 1455 CD GLU 181 18.738 -6.511 89.342 1.00 47.46
ATOM 1456 OE1 GLU 181 17.501 -6.522 89.412 1.00 47.46
ATOM 1457 OE2 GLU 181 19.419 -5.589 89.823 1.00 47.46
ATOM 1458 C GLU 181 19.125 -10.230 87.096 1.00 35.90
ATOM 1459 O GLU 181 19.771 -10.892 87.910 1.00 35.90
ATOM 1460 N ILE 182 17.899 -10.555 86.709 1.00 38.32
ATOM 1461 CA ILE 182 17.210 -11.717 87.243 1.00 38.32

ATOM 1462 CB ILE 182 15.724 -11.673 86.870 1.00 31.34
ATOM 1463 CG2 ILE 182 15.056 -13.010 87.194 1.00 31.34
ATOM 1464 CG1 ILE 182 15.051 -10.513 87.595 1.00 31.34
ATOM 1465 CD1 ILE 182 13.680 -10.173 87.047 1.00 31.34
ATOM 1466 C ILE 182 17.798 -13.040 86.773 1.00 38.32
ATOM 1467 O ILE 182 17.841 -13.997 87.541 1.00 38.32
ATOM 1468 N MET 183 18.249 -13.094 85.520 1.00 47.57
ATOM 1469 CA MET 183 18.819 -14.318 84.957 1.00 47.57
ATOM 1470 CB MET 183 18.614 -14.370 83.444 1.00 45.73
ATOM 1471 CG MET 183 17.174 -14.221 82.994 1.00 45.73
ATOM 1472 SD MET 183 16.076 -15.382 83.795 1.00 45.73
ATOM 1473 CE MET 183 16.837 -16.936 83.323 1.00 45.73
ATOM 1474 C MET 183 20.299 -14.434 85.261 1.00 47.57
ATOM 1475 O MET 183 20.777 -15.516 85.583 1.00 47.57
ATOM 1476 N GLU 184 21.026 -13.325 85.128 1.00 64.62
ATOM 1477 CA GLU 184 22.455 -13.295 85.437 1.00 64.62
ATOM 1478 CB GLU 184 23.104 -12.016 84.885 1.00100.23
ATOM 1479 CG GLU 184 22.781 -11.669 83.422 1.00100.23
ATOM 1480 CD GLU 184 23.213 -12.733 82.429 1.00100.23
ATOM 1481 OE1 GLU 184 24.282 -13.344 82.642 1.00100.23
ATOM 1482 OE2 GLU 184 22.490 -12.945 81.426 1.00100.23
ATOM 1483 C GLU 184 22.528 -13.313 86.974 1.00 64.62
ATOM 1484 O GLU 184 23.295 -12.582 87.596 1.00 64.62
ATOM 1485 N PHE 185 21.677 -14.170 87.538 1.00 64.82

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ATOM   1486  CA  PHE   185      21.470 -14.427  88.968  1.00 64.82
ATOM   1487  CB  PHE   185      21.845 -15.885  89.308  1.00100.63
ATOM   1488  CG  PHE   185      23.303 -16.226  89.133  1.00100.63
ATOM   1489  CD1 PHE   185      24.222 -15.967  90.146  1.00100.63
ATOM   1490  CD2 PHE   185      23.744 -16.877  87.984  1.00100.63
ATOM   1491  CE1 PHE   185      25.554 -16.363  90.018  1.00100.63
ATOM   1492  CE2 PHE   185      25.074 -17.272  87.854  1.00100.63
ATOM   1493  CZ  PHE   185      25.975 -17.015  88.872  1.00100.63
ATOM   1494  C   PHE   185      22.019 -13.492  90.040  1.00 64.82
ATOM   1495  O   PHE   185      21.202 -13.108  90.910  1.00 64.82
ATOM   1496  OT  PHE   185      23.222 -13.161  90.023  1.00100.63
ATOM   1497  OH2 WAT     1       7.314  -7.392 118.599  1.00 28.51
ATOM   1498  OH2 WAT     2      20.141 -10.167 116.646  1.00 41.71
ATOM   1499  OH2 WAT     3      -0.839   7.103  71.695  1.00 38.40
ATOM   1500  OH2 WAT     4      20.724 -12.318 113.141  1.00 36.06
ATOM   1501  OH2 WAT     5      13.214   7.193 109.995  1.00 34.42
ATOM   1502  OH2 WAT     6      -4.563  25.067  61.299  1.00 35.61
ATOM   1503  OH2 WAT     7       9.162 -10.160  92.783  1.00 40.02
ATOM   1504  OH2 WAT     8      15.821   7.078  82.761  1.00 45.31
ATOM   1505  OH2 WAT     9      15.568   0.183  88.102  1.00 24.68
ATOM   1506  OH2 WAT    10      17.031  -3.108  93.557  1.00 33.93
ATOM   1507  OH2 WAT    11      -5.066  12.003  70.605  1.00 31.32
ATOM   1508  OH2 WAT    12      17.672 -16.020 114.048  1.00 36.87
ATOM   1509  OH2 WAT    13       7.862  -1.817  71.942  1.00 33.70
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ATOM   1569  OH2 WAT    73       3.860  -5.877  90.214  1.00 44.03
```

CLAIMS

1. A method for designing a compound capable of binding to an active site, an accessory binding site or a pocket of an RRF protein, which comprises computationally evaluating a chemical entity of RRF protein on the basis of a structure coordinate obtained from an RRF protein crystal.

2. The method according to claim 1, wherein the RRF protein crystal is any crystal of the RRF protein itself, an RRF protein mutant, an RRF protein homologue or an RRF protein co-complex.

3. The method according to claim 1 or 2, wherein the RRF protein crystal is bipyramidal.

4. The method according to any one of claims 1 to 3, wherein the RRF protein crystal has a space group $P4_12_12_1$ or a space group $P4_32_12$.

5. The method according to any one of claims 1 to 4, wherein the RRF protein crystal has a size of 0.3 × 0.3 × 0.5 mm.

6. The method according to any one of claims 1 to 5, wherein the RRF protein crystal has respective unit lattices of a size of a=b=47.3Å and c=297.6Å.

7. The method according to any one of claims 1 to 6, wherein the RRF protein crystal is characterized by a structure coordinate described in Table 7.

8. The method according to claims 1 to 7, wherein the RRF protein crystal is derived from Thermotoga Maritima.

9. The method according to any one of claim 1 or 2, wherein the RRF protein crystal is orthorhombic.

10. The method according to any one of claims 1, 2 and 9, wherein the RRF protein crystal has a space group $P2_12_12$.

11. The method according to any one of claims 1 to 2 and 9 to 10, wherein the RRF protein crystal has a size of 30 × 50 × 250 μm.

12. The method according to any one of claims 1 to 2 and 9 to 11, wherein the RRF protein crystal is derived from strain X.

13. The method according to any one of claims 1 to 12, wherein the RRF protein crystal is crystallized by a drop-like vapour diffusion method.

14. The method according to any one of claims 1 to 13, wherein the RRF protein crystal is a heavy atom derivative and the crystal is any crystal of the RRF protein itself, an RRF protein mutant, an RRF protein homologue or an RRF protein co-complex.

15. The method according to any one of claims 1 to 14, wherein the heavy atom derivative is formed by reaction of a compound selected from the group consisting of thyromethal, gold thiomalate, uranyl acetate and lead chloride.

16. The method according to any one of claims 1, 2 and 9 to 12, wherein the RRF protein crystal is a heavy atom derivative of platinum or mercury.

17. The method according to any one of claims 1 to 16, wherein the RRF protein is a monomer.

18. The method according to any one of claims 1 to 8, 13 to 15 and 17, wherein the RRF protein is characterized by amino acid displacement according to Table 5 or Table 6.

19. The method according to any one of claims 1 to 18, wherein a compound characterized by the chemical entity bound to the active site, accessory binding site or pocket is an inhibitor to the RRF

protein.

20. The method according to any one of claims 1 to 19, wherein the inhibitor is a competitive inhibitor, an uncompetitive inhibitor or a noncompetitive inhibitor to the RRF.

21. The method according to any one of claims 1 to 20, comprising determining orientation of a ligand at the active site or accessory binding site of the RRF protein.

22. The method according to any one of claims 1 to 8, 13 to 15 and 17 to 21, wherein the structure coordinate is a structure coordinate of the RRF protein according to Table 7.

23. A method for determining a three-dimensional structure of an RRF protein, comprising elucidating crystal form of a mutant, homologue or co-complex of the RRF protein by molecular replacement.

24. An RRF protein crystal which is orthorhombic.

25. The RRF protein crystal according to claim 24, having a space group $P2_12_12$.

26. The RRF protein crystal according to claim 24 or 25, having

a size of 30 × 50 × 250 μm.

27. The RRF protein crystal according to any one of claims 24 to 26, wherein the RRF is derived from strain X.

28. The RRF protein crystal which is bipyramidal.

29. The RRF protein crystal according to claim 28, wherein the RRF protein crystal has a space group $P4_12_12_1$ or a space group $P4_32_12$.

30. The RRF protein crystal according to claim 28 or 29, having a size of 0.3 × 0.3 × 0.5 mm.

31. The RRF protein crystal according to any one of claims 28 to 30, having respective unit lattices of a size of a=b=47.3Å and c=297.6Å.

32. The RRF protein crystal according to any one of claims 28 to 31, characterized by amino acid displacement according to Table 5 or Table 6.

33. The RRF protein crystal according to any one of claims 28 to 32, characterized by a structure coordinate according to Table 7.

34. The RRF protein crystal according to any one of claims 28 to 33, derived from Thermotoga Maritima.

35. The RRF protein crystal according to any one of claims 24 to 34, crystallized by a drop-like vapour diffusion method.

36. The RRF protein crystal according to any one of claims 24 to 35, wherein the crystal is any crystal of the RRF protein itself, an RRF protein mutant, an RRF protein homologue or an RRF protein co-complex.

37. An RRF protein, wherein amino acid in an active site is selected from the group consisting of Arg 110, Arg 129 and Arg 132 of SEQ. ID. NO. 1.

38. The RRF protein according to claim 37, wherein at least one amino acid in the active site or accessory active site is replaced by at least one amino acid selected from the group consisting of naturally occurring amino acids, non-natural amino acids, selenocysteine and selenomethionine.

39. The RRF protein according to claim 37, wherein a hydrophilic amino acid or a hydrophobic amino acid in the active site or accessory active site is replaced.

40. The RRF protein according to claim 37, wherein at least one cysteine amino acid is replaced by an amino acid selected from the group consisting of selenocysteine and selenomethionine.

41. The RRF protein according to claim 37, wherein at least one methionine amino acid is replaced by an amino acid selected from the group consisting of selenocysteine or selenomethionine.

42. The RRF protein according to any one of claims 37 to 38, wherein the RRF protein is in a crystal form.

43. The RRF protein according to claim 37, having a specific activity higher or lower than that of a wild type enzyme.

44. The RRF protein according to claim 37, having a varied substrate specificity.

45. Use of an RRF protein according to claim 37 for measuring binding interaction between a compound and the RRF protein.

46. The RRF protein according to claim 37, wherein at lest one amino acid residue on a surface of the RRF protein, in the surface or in the vicinity thereof is replaced and a change in surface charge

by one or more charge units occurs.

47. The method according to any one of claims 1 to 22, wherein the pocket of the RRF protein is a pocket in the vicinity of C-terminal positioned on a folded part separating two domains of the RRF protein.

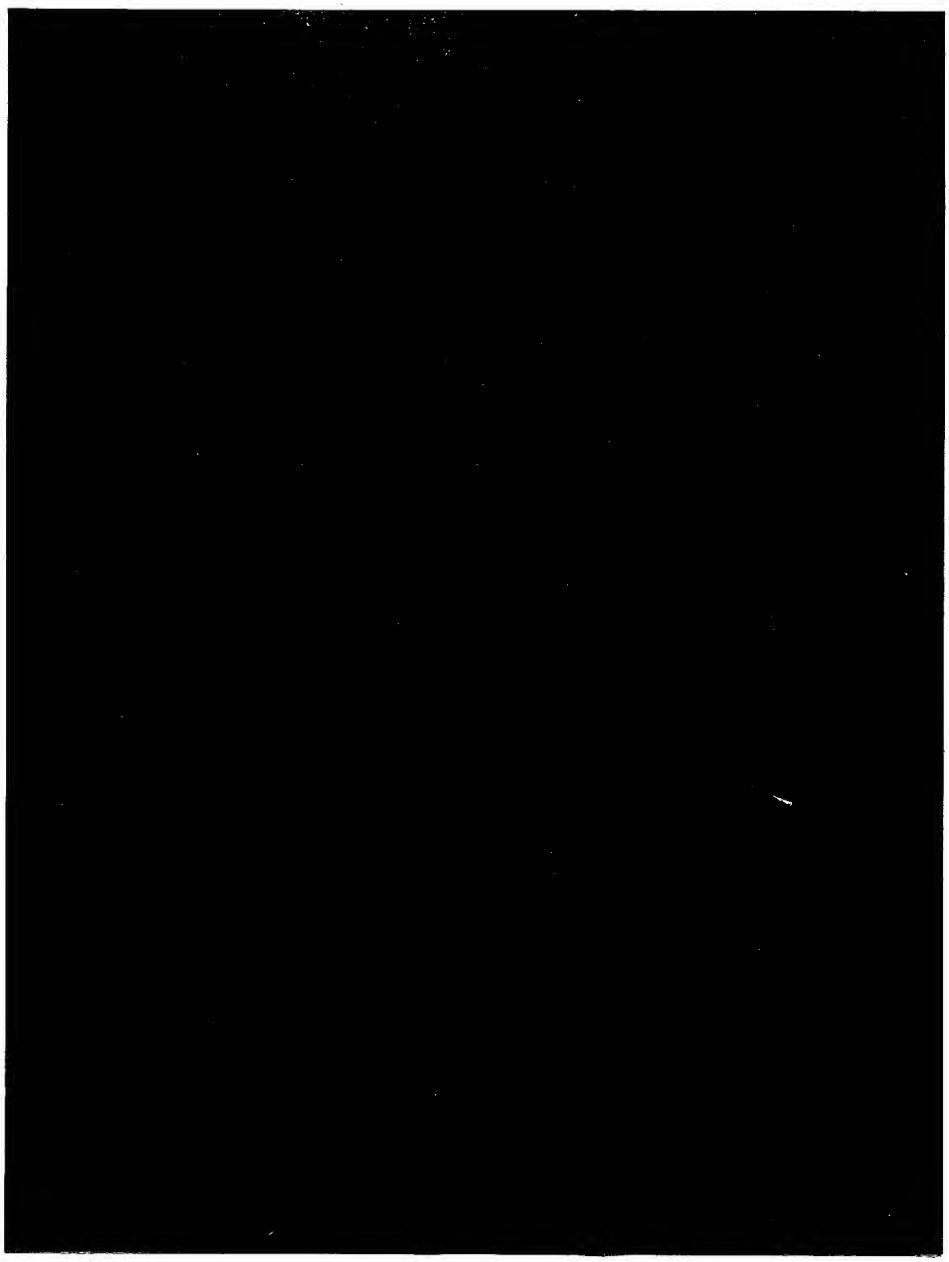
48. The method according to any one of claims 1 to 22, wherein the compound inhibits binding of the RRF protein to ribosome or inhibits behavior of the RRF protein on the ribosome.

49. The inhibitor to an RRF protein, obtained by the method according to any one of claims 19 to 23, 47 and 48.

50. A method for searching a compound that can inhibit activity of an RRF protein on the basis of its activity of inhibiting binding of the RRF protein to ribosome or its activity of inhibiting behavior of the RRF protein on the ribosome.

51. An inhibitor to an RRF protein, obtained by the method according to claim 50.

Fig. 1



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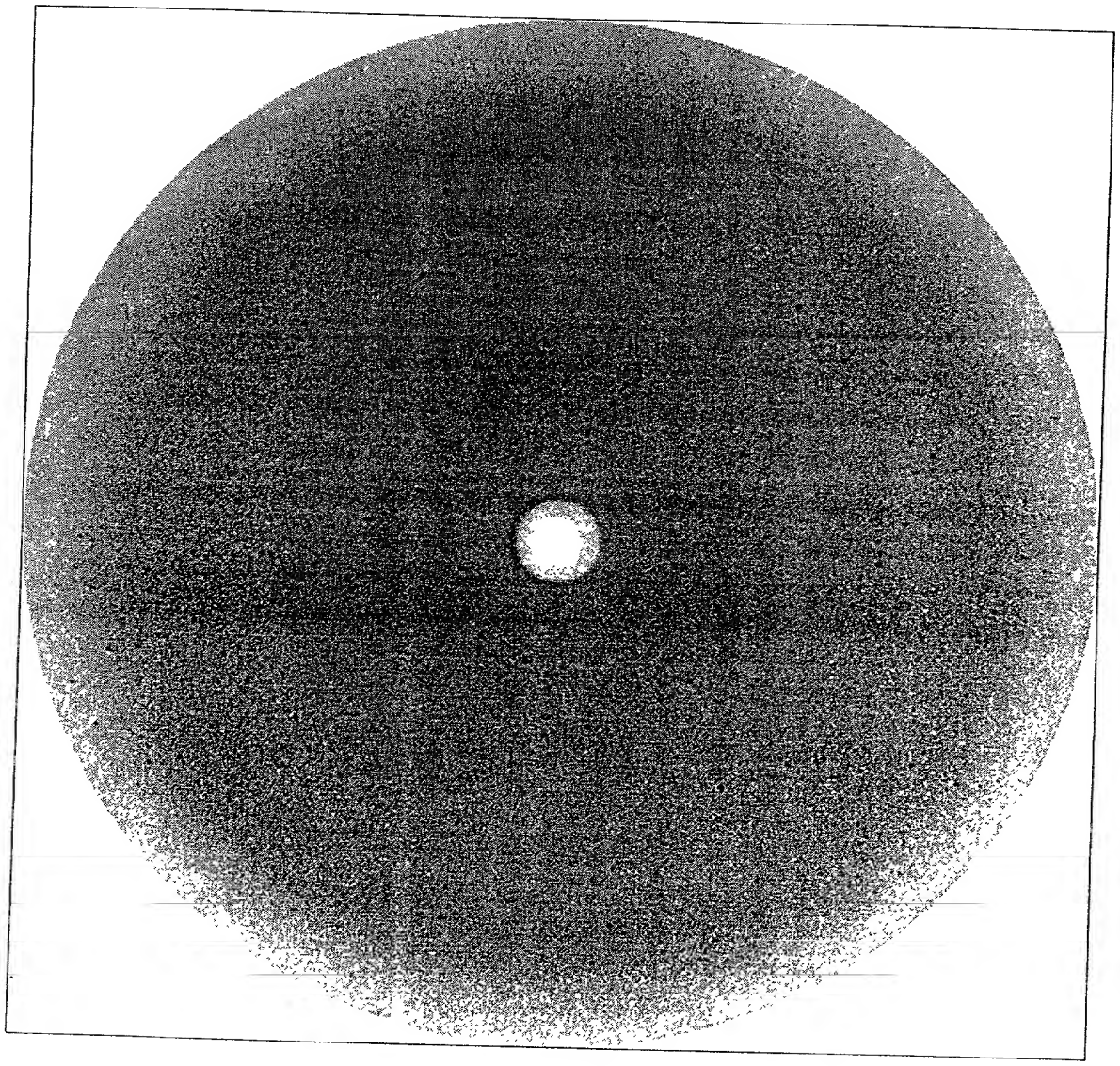

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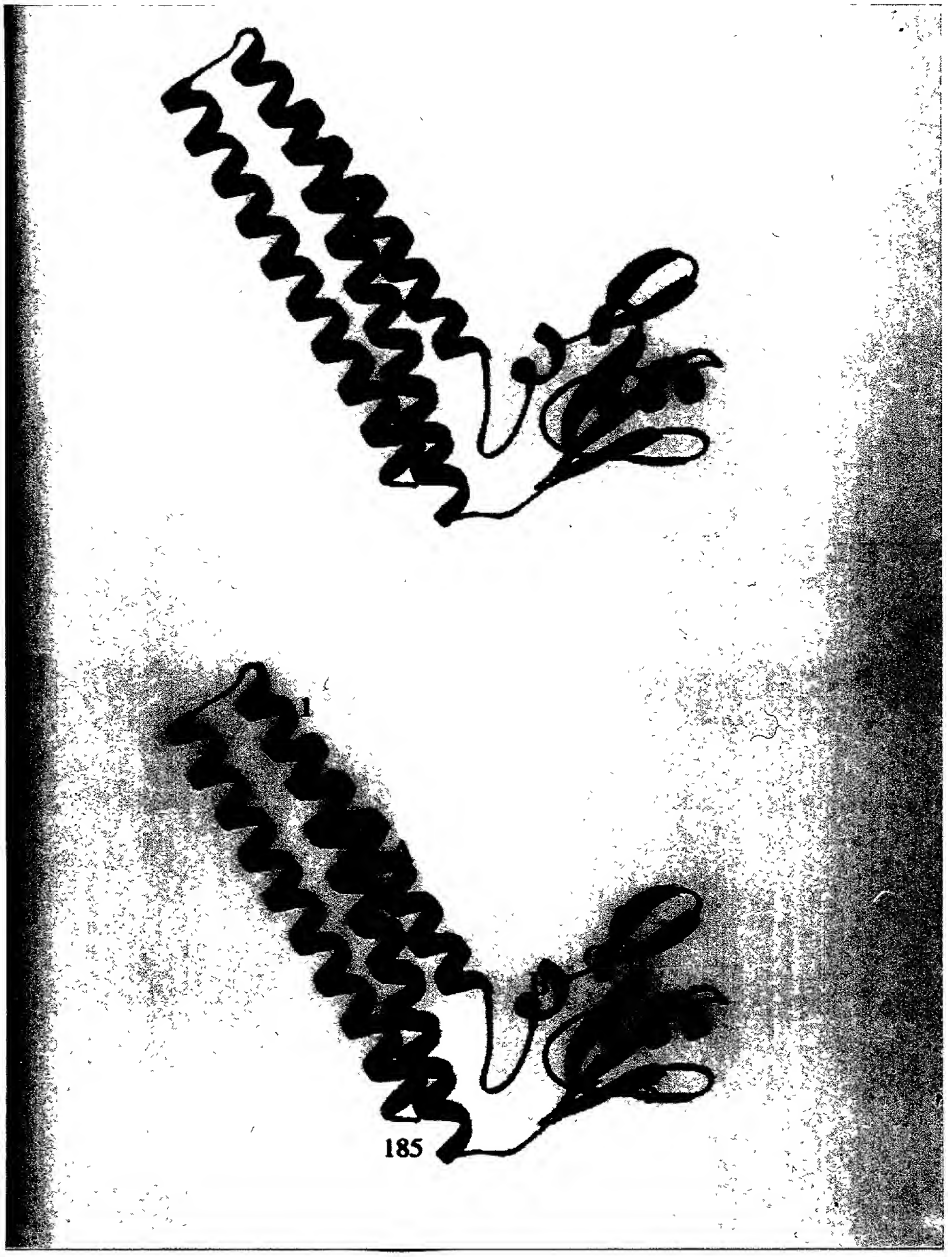

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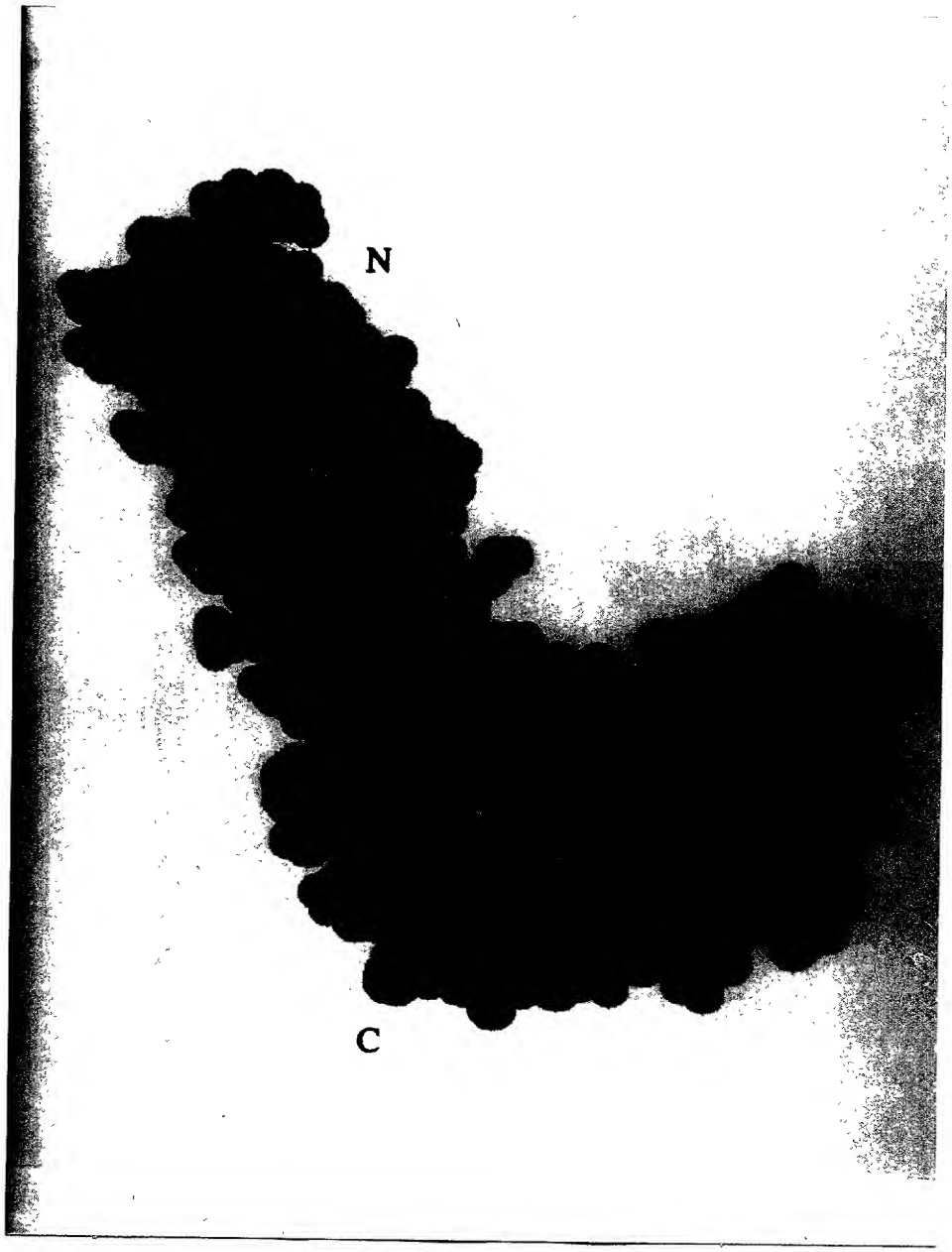

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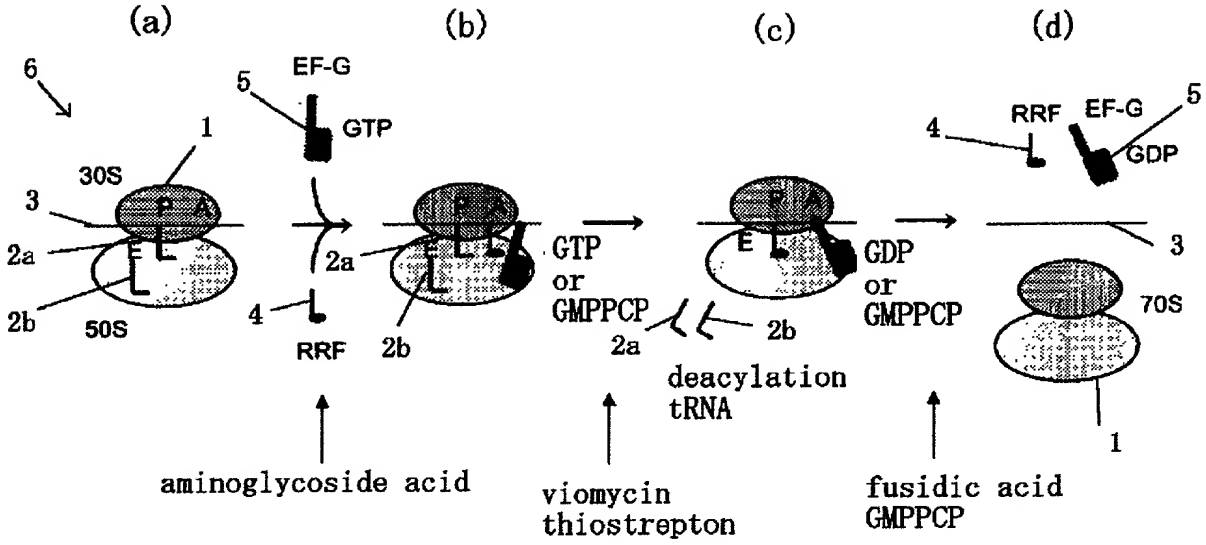
(a)
(b)
(c)
(d)
6
5
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GTP
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2b
50S
4
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GTP
or
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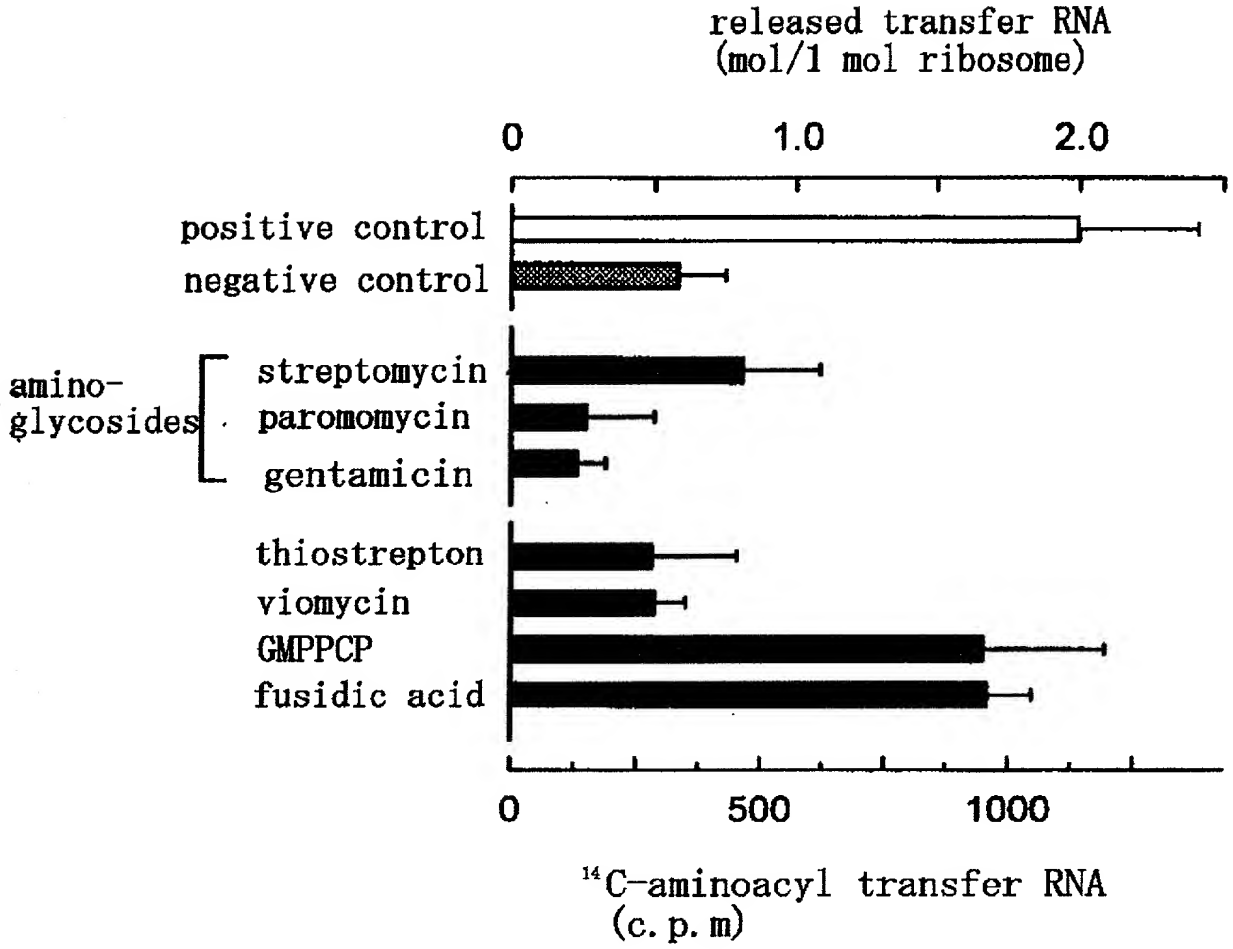
Fig. 6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2.0
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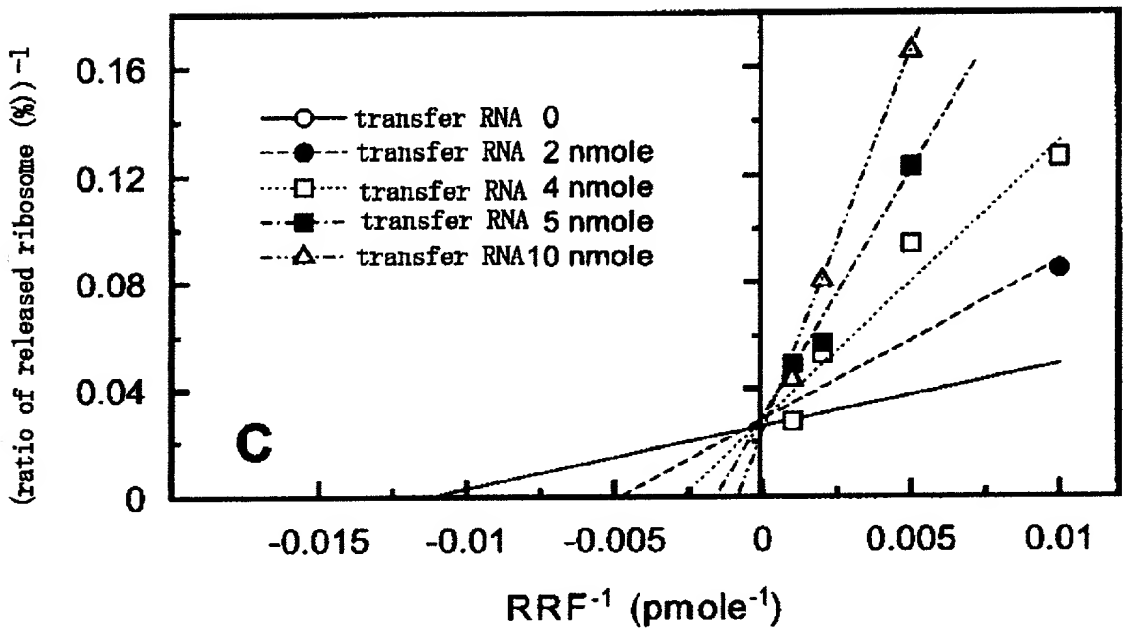
Fig. 7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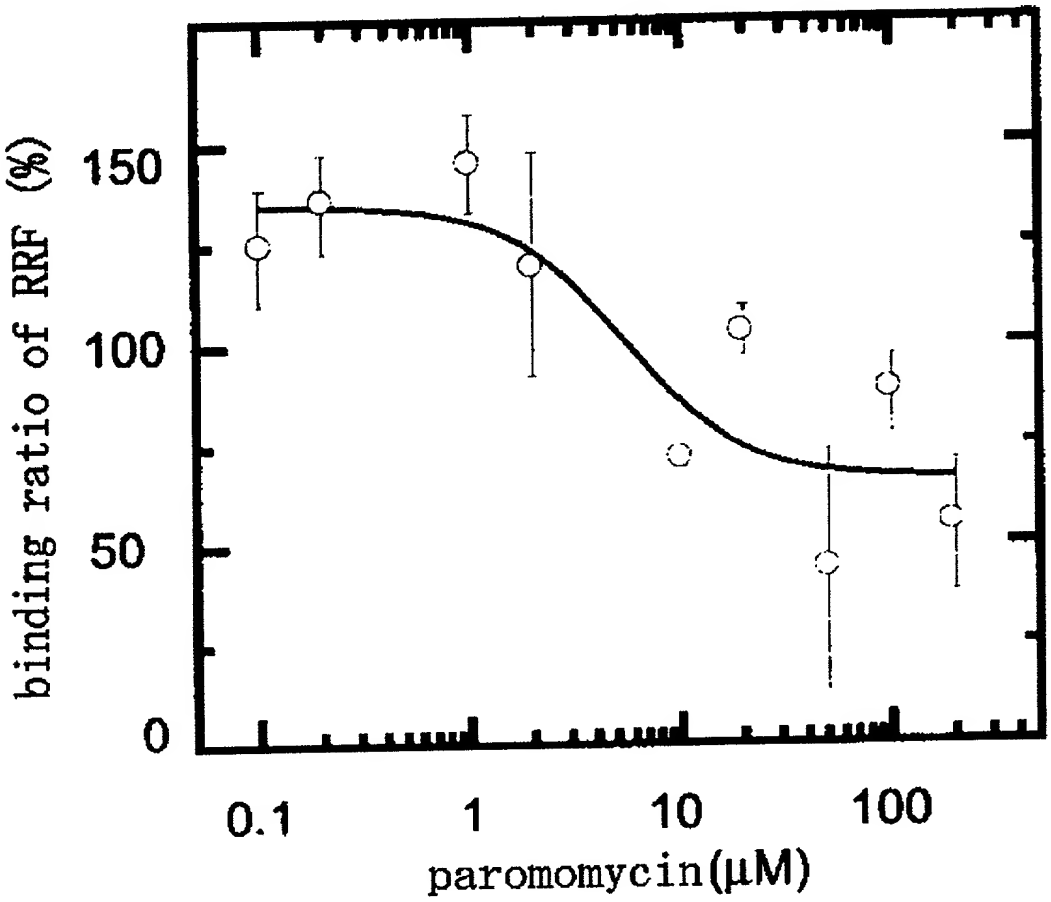
0.01
RRF^{-1} (pmole^{-1})

Fig. 8



Attorney Docket No. **K0448/7012**

DECLARATION FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled:

CRYSTAL OF RIBOSOMAL RECYCLING FACTOR (RRF) PROTEIN AND APPLICATION THEREOF ON THE BASIS OF THREE-DIMENSIONAL STRUCTURAL DATA OBTAINED FROM THE CRYSTAL

the specification of which is attached hereto unless the following is checked:

[X] was filed on June 5, 2000, as PCT International Application No.PCT/JP00/03639, bearing attorney docket No. K0448/7012.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or section 365(a) of any PCT International application designating at least one country other than the United States listed below and have also identified below any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed:

Prior Foreign PCT International Application(s)and any priority claims under 35 U.S.C. §§119 and 365(a),(b):

			Priority Claimed
11-158637 (Number)	**Japan** (Country-if PCT, so indicate)	**04/06/99** (DD/MM/YY Filed)	[X] [ ] YES NO
_____ (Number)	_____ (Country-if PCT, so indicate)	_____ (DD/MM/YY Filed)	[ ] [ ] YES NO
_____ (Number)	_____ (Country-if PCT, so indicate)	_____ (DD/MM/YY Filed)	[ ] [ ] YES NO

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below:

(Application Number)	(filing date)

(Application Number) (filing date)

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s), or §365(c) of any PCT International application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

(Application No.)	(filing date)	(status-patented, pending, abandoned)
(Application No.)	(filing date)	(status-patented, pending, abandoned)

PCT International Applications designating the United States:

(PCT Appl. No.) (U.S. Ser. No.) (PCT filing date) (status-patented,pending,abandoned)

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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Thomas G. Field	45,596	Helen C. Lockhart	39,248	Paul D. Sorkin	39,039
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Edward R. Gates	31,616	William R. McClellan	29,409	Mark Steinberg	40,828
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Lawrence M. Green	29,384	Edward F. Perlman	28,105	Lisa E. Winsor	44,405
George L. Greenfield	17,756	Michael J. Pomianek	46,190	David Wolf	17,528
James M. Hanifin, Jr.	39,213	Elizabeth R. Plumer	36,637	Douglas R. Wolf	36,971
Therese A. Hendricks	30,389	Randy J. Pritzker	35,986	Ivan D. Zitkovsky	37,482
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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment,

or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Akira Kaji — Jan 7, 02

Inventor's signature — Date

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SEQUENCE LISTING

<110> KAJI, Akira
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<120> CRYSTAL OF RIBOSOMAL RECYCLING FACTOR (RRF) PROTEIN AND APPLICATION THEREOF ON THE BASIS OF THREE-DIMENSIONAL STRUCTURAL DATA OBTAINED FROM THE CRYSTAL

<130> K0448/7012 (JRV)

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